

1962

Biology and morphology of *Demodex canis*

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BIOLOGY AND MORPHOLOGY OF DEMODEX
CANIS.

Iowa State University of Science and Technology
Ph.D., 1962
Zoology

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BIOLOGY AND MORPHOLOGY OF DEMODEX CANIS

by

Frank Elwood French, Jr.

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

Signature was redacted for privacy.

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Iowa State University
Of Science and Technology
Ames, Iowa

1962

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TABLE OF CONTENTS

| | Page |
|---|------|
| GENERAL INTRODUCTION | 1 |
| PART I. MORPHOLOGY OF <u>DEMODEX CANIS</u> | 3 |
| INTRODUCTION | 4 |
| METHODS AND MATERIALS | 6 |
| OBSERVATIONS, DESCRIPTIONS, AND DISCUSSION | 7 |
| SUMMARY | 26 |
| PART II. <u>DEMODEX CANIS</u> IN INTERNAL TISSUES | 27 |
| INTRODUCTION | 28 |
| METHODS AND PROCEDURES | 32 |
| OBSERVATIONS AND DISCUSSION | 38 |
| SUMMARY | 68 |
| PART III. TRANSMISSION OF <u>DEMODEX CANIS</u> | 70 |
| INTRODUCTION | 71 |
| METHODS AND MATERIALS | 72 |
| INVESTIGATIONS | 75 |
| SUMMARY | 92 |
| LITERATURE CITED | 94 |
| ACKNOWLEDGMENTS | 98 |
| APPENDIX | 99 |

GENERAL INTRODUCTION

Since the description of Demodex canis Leydig, 1859, this mite and the associated dermatitis, demodectic mange, have been discussed in more than 500 publications. The skin condition has attracted the most attention.

The mites, D. canis, are located primarily in the hair follicles, occasionally in the sebaceous and sweat glands. The mites are also found in various internal organs and tissues, especially in superficial lymph nodes.

The dermatitis, known as demodectic mange, varies greatly in gross appearance. Demodectic mange with little secondary bacterial infection is commonly called the squamous form. The gross lesions may range from small, scattered, hairless spots to extensive atrichia, involving almost the entire body, with hyperplasia of the epithelium and connective tissue. Secondary bacterial invasion produces a condition known as the pustular form of demodectic mange. Reactions include hyperplasia, swelling associated with suppurative inflammation, emaciation, and general edema.

Controversy exists as to whether the mite, D. canis, causes the dermatitis or whether the skin condition allows a large population of mites to exist. The truth probably lies somewhere between these two theses. Král (1960, page 55) stated:

Etiologically, demodectic mange in dogs has long been considered to be a true infectious disease produced by hair-follicle mites. Recent

experimental research results have shown, however, that Demodex mites, as such, appear to be of little consequence in dogs which are in a perfect state of health. It is not until the animal is subjected to various devitalizing factors, such as nutritional deficiencies, internal parasites, systemic disorders, etc., that these parasites become a serious problem. It is only then that the hair-follicle mites and secondarily, bacteria (Staphylococcus pyogenes) can bring about the development of demodectic mange.

Runnells et al. (1960, page 704) wrote:

There is some question as to just how much damage in the skin is due to allergic manifestations and bacterial infection and how much is due to the mites. If a suppurative inflammation, indicating the presence of bacteria, is not present, it is surprising how little reaction is actually found in the skin.

Many therapeutic measures have been proposed for demodectic mange, some of which are discussed by Hirst (1919), Koutz (1955), and Král (1960). A dermatologist, Král (1960, page 57), wrote: ". . . at this time there is still no specific and reliable treatment for demodectic mange in dogs."

More basic information on D. canis and its relationship to the host should assist attempts to devise reliable treatments for demodectic mange. This dissertation is concerned with the morphology and stadia of D. canis, mode of transmission, and the presence of the mites in lymph nodes and other internal organs.

PART I. MORPHOLOGY OF DEMODEX CANIS

INTRODUCTION

The Demodicidae are parasites of mammals. The genus Demodex was created by Owen (1843) for the human follicle mite, D. folliculorum (Simon) 1842. Species of Demodex have been described from the skin of Primates, Insectivora, Rodentia, Lagomorpha, Carnivora, Perissodactyla, and Artiodactyla. Fain (1959) described two new genera, Rhinodex and Stomatodex. Rhinodex were found in the nasal fossae of Dermoptera. Species of Stomatodex were described from the buccal mucosa of Dermoptera and Chiroptera (Fain, 1959 and 1960).

Hirst (1919) discussed the origin of Demodicidae and relationships to other Acarina. He concluded that the Demodicidae were a degenerate branch of the Cheyletidae based on the structure of the penis and the anterior dorsal position of the male genital opening. He also noted similarity to the Myobiidae and Harpyrhynchidae.

Demodex canis was described and figured by Leydig (1859). Mégnin (1877) also described and figured D. canis. Hirst (1919), in his masterful monograph on Demodex, discussed D. canis briefly and gave figures for the female. He did not discuss the immature stages of D. canis; however a detailed account was given for immature stages of D. muscardini Hirst.

Hirst (1919) listed the following stages for Demodex: ovum, larva, protonymph, deutonymph, female, and male. Sokolovskii (1952) described the stadia of D. canis as ovum,

larva, protonymph, deutonymph, female, and male. Sokolovskii contended that the deutonymphal stage could be omitted when conditions were unfavorable for the mites. His paper was illustrated with photomicrographs but no drawings.

METHODS AND MATERIALS

Specimens were mounted on glass slides in Hoyer's modification of Berlese's solution and in polyvinyl alcohol. The formulae for the media are given in the Appendix under Mounting Media. The polyvinyl alcohol was the best general purpose medium and was most efficient when the specimens were mounted directly from 75 to 80% ethyl alcohol. Some specimens were cleared by 6% potassium hydroxide or "acid chloral hydrate" (40 g chloral hydrate, 25 ml distilled water, and 2.5 ml hydrochloric acid) before mounting in Hoyer's modification of Berlese's solution.

The specimens were observed with a phase-contrast microscope¹ equipped with 10X, 20X, 43X, and 97X dark-contrast objectives and a 97X bright-contrast objective. The drawings were made with the aid of a camera lucida.

¹A-O Phasestar, American Optical Company, Buffalo, New York.

OBSERVATIONS, DESCRIPTIONS, AND DISCUSSION

Ovum

The ova of D. canis are characteristically described as spindle shaped. The length may range from 80 to 105 μ . The greatest width varied from 32 to 54 μ . In many ova, a proto-larval form was identified within the eggshell (Fig. 8).

In contrast to the reports of Hirst (1919) and other authors on various species of Demodex, I identified eggshells within females in less than 1% of the observed specimens of D. canis (Fig. 21). Masses of cells, apparently developing ova, were noted in nearly all females. Perhaps the eggshell is formed very shortly before oviposition. Newly formed shells, perhaps, were unable to withstand the clearing action of chloral hydrate, lactic acid, or potassium hydroxide.

Protolarva

Two morphologically distinct six-legged immature forms or stages were observed. I designated the forms that emerge from ova as protolarvae of Demodex canis Leydig (Fig. 1). The stage that succeeds the protolarva was designated the deutolarva (Fig. 2). It is generally stated in the literature that the larvae possess three pairs of legs or appendages while the nymphal and adult stages of Demodex have four pairs of legs. These criteria are in accordance with the monographic work of Hirst (1919) and Sokolovskii (1952) on D.

canis. Apparently the only criterion, other than the number of legs, for the larval stage is that the larva directly succeeds the ovum. The use of "deutolarva" was less confusing than referring to the stage as a six-legged nymph.

Description

Three pairs of very short and weakly sclerotized legs or appendages are present. These appendages appear to consist chiefly of a distal plate and a more or less conical basal segment. Each distal plate bears a single tridentate tubercle. The tubercles are less than $2\ \mu$ in length and are represented by Figs. 3 and 4. Hirst (1919) referred to the distal podal plate as the epimeron, but it seems best to abandon this entomological term in acarology.

Medio-ventral podosomal discs are not present.

A short bifid spine is present on the dorsum of the basal pedipalpal segment. The spines are less than $2\ \mu$ in length (Figs. 5 and 6). The distal segment or plate of the pedipalp bears three short and sharply pointed spines as illustrated in Fig. 1.

The length, width and shape of the mites vary considerably. Some of the size variability is undoubtedly due to the stage of development, i.e. whether recently emerged from an ovum or nearing the deutolarval stage. Also, some variance in size was the result of different mounting media and pressures by the cover glasses on the specimens. Fig. 1

represents a protolarva that had recently emerged from an ovum. The average length of six specimens measured was about 120 μ . The shortest specimen was 115 μ while the longest measured 130 μ . The average width, measured between the tubercles of the second appendages, was 36 μ and the range was 33 to 37 μ . The distance between the tubercles of appendage I and appendage III varied from 24 to 31 μ , average 26 μ .

The protolarva has fine annular striations on the opisthosoma and extending over the dorsal podosomal region. The striations are less pronounced than in the deutolarval, nymphal, and adult stadia.

Deutolarva

Description

This stage differs morphologically from the protolarva primarily in two respects. The basal podal plates of the three pairs of appendages bear two tridentate tubercles each. The pairs of tubercles are of the same shape and magnitude as the single tubercles found on the protolarva.

Three pairs of semicircular podosomal discs, projected posteriorly, are found on the medio-ventral podosoma. One pair of podosomal discs is located between each of the three pairs of appendages (Fig. 2). Fig. 7 represents a lateral view of a sagittal section of a podosomal disc.

The gnathosoma is quite similar to that of the proto-

larva. No measurable differences were detected between the pedipalpal spines of the protolarva and the deutolarva.

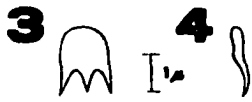
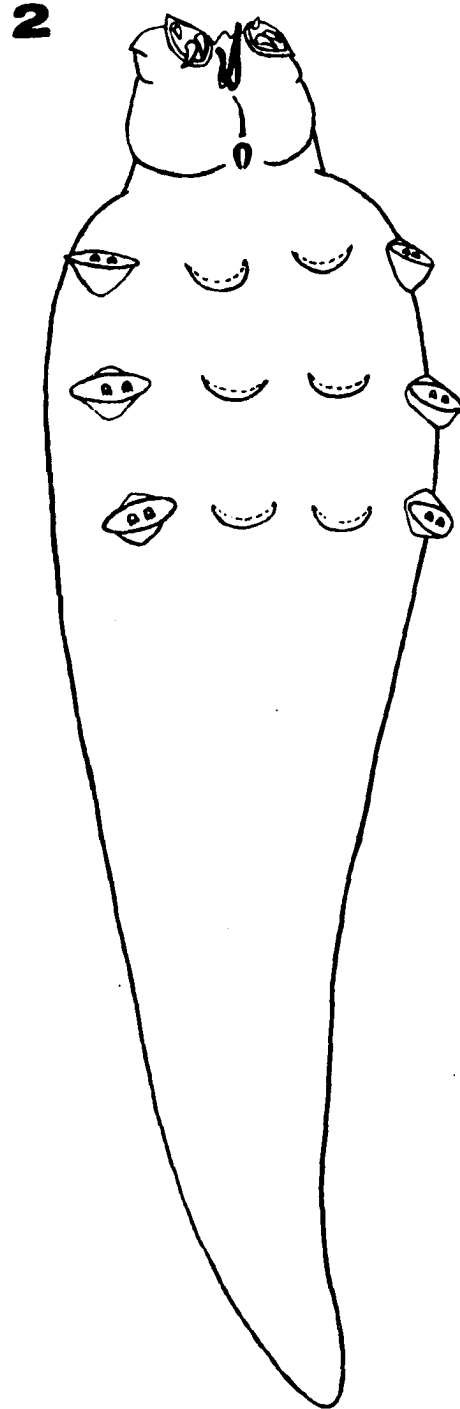
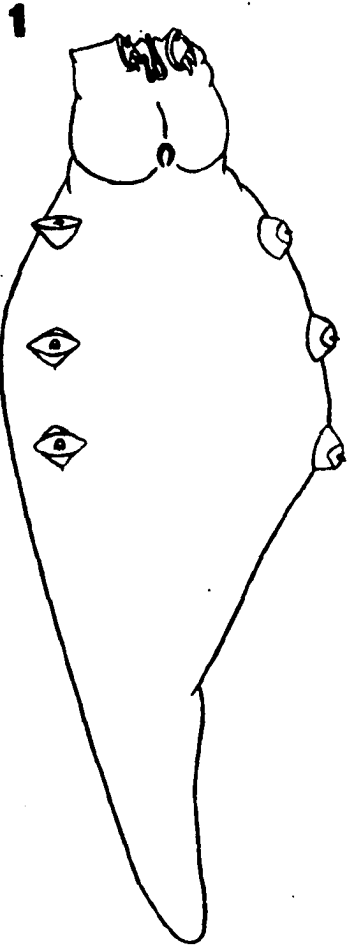
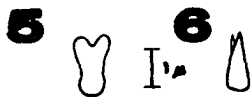
The size and shape of the deutolarvae are quite variable and are of little taxonomic value. Fig. 2 represents a deutolarva that would soon molt into a protonymph. The total length ranged from 140 to 180 μ in eight specimens. The distance between the innermost tubercles of legs II ranged from 34 to 39 μ . The distance from the tubercles of legs I to III varied from 29 to 36 μ .

Discussion

Mégnin (1877) gave measurements for a first larva (apodous) and a second larva (hexapodous) of D. canis. For D. folliculorum, Mégnin reported measurements for two types of apodous larvae and a hexapodous larva. Unfortunately, the drawings representing the larval stages lacked detail and were little more than outlines of body shapes.

The beautifully illustrated monograph of Hirst (1919) primarily contained descriptions of adult Demodex. His paper did not have an illustration or a description of a larval D. canis. However, Hirst included a detailed drawing of a larval D. folliculorum with two tridentate tubercles on each of the six legs. Also, he depicted an appendage of a larval D. folliculorum with a single tubercle. Hirst (1919, page 21) stated in reference to D. folliculorum, "Smaller specimens of this slender larval form apparently sometimes

- Fig. 1. Protolarva of Demodex canis Leydig, ventral view
- Fig. 2. Deutolarva of D. canis, ventral view
- Fig. 3. Tubercle of distal podal plate, ventral view, larval D. canis
- Fig. 4. Tubercle of distal podal plate, lateral view, larval D. canis
- Fig. 5. Dorsal spine of basal pedipalpal segment, anterior view, larval D. canis
- Fig. 6. Dorsal spine of basal pedipalpal segment, lateral view, larval D. canis
- Fig. 7. Ventral podosomal disc of deutolarval D. canis, sagittal section

I₁I₂I₁

have only a single median tubercle on the epimeron instead of two." Thus, Hirst may have observed two larval stages of D. folliculorum similar to the protolarva and deutolarva of D. canis described herein. Sokolovskii (1952) stated that sexual dimorphism was not evident in the larval stages and made no mention of two types of larvae with morphologically distinct integuments. He stated the larvae had three pairs of legs but did not mention tubercles on the legs or ventral podosomal discs.

I observed two, six-legged immature forms in skin samples from dogs, Canis familiaris L. Upon discovery of the two types of larvae, three explanations seemed possible. First, there was the possibility of sexual dimorphism in the larval stage. Second, it was possible that two species of Demodex were present on the host dogs. Lastly, there was the possibility of two larval stages, i.e. protolarva and deutolarva. After discovery of eight protolarvae each of which had a deutolarval form within, the first and second possibilities were abandoned. Fig. 9 is a photomicrograph of a protolarval integument with a deutolarva within. It appears that indeed there are two larval stadia and that the first (protolarval) produces the second stage (deutolarval). Protolarval forms could be distinguished within 79 of 278 ova in one sample. At no time was a deutolarval form observed within an ovum. Numerous protonymphs were observed to be casting deutolarval skins and several deutolarvae were observed con-

Fig. 8. Ovum of Demodex canis with developing protolarva
(phase-contrast photomicrograph)

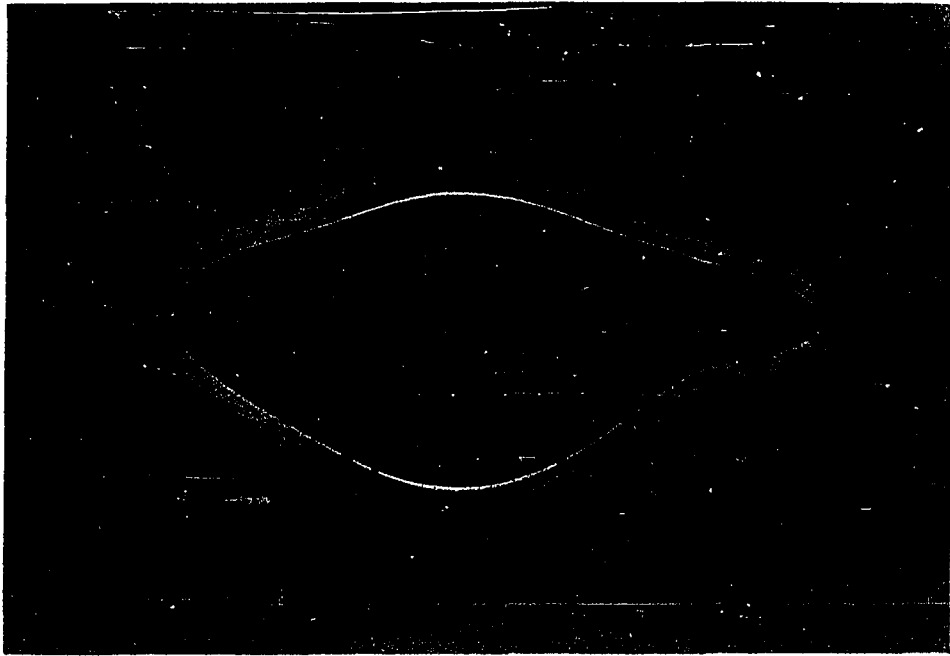
A. Tubercle of distal podal plate, leg I, protolarva

Fig. 9. Protolarva of D. canis shortly before molting,
deutolarval integument within (phase-contrast
photomicrograph)

A. Tubercle, leg II of protolarva

B. Tubercles, leg II of deutolarval integument
within the protolarva

C. Ventral podosomal disc III of deutolarva



taining protonymphs within their integuments. No protolarvae were observed molting directly to the protonymphal stage; whereas, eight protolarvae were observed to contain the deutolarval stage. Specimens of protolarvae, deutolarvae, and molting protolarvae will be deposited in the United States National Museum.

Protonymph

The protonymph bears a striking resemblance to the deutolarva. The only distinct differences are the number of appendages and podosomal discs.

Description

The four pairs of appendages bear two tridentate tubercles each (Fig. 10). No measurable difference was detected between the tubercles of the protonymph and larvae.

Four pairs of semicircular discs are present on the medio-ventral podosoma.

The gnathosoma is essentially the same as in the larvae. No measurable differences were detected between the pedipalpal spines or chelicerae of the protolarva, deutolarva, and protonymph. The dorsal bifid spine on the basal pedipalpal segment is the same as shown for the larvae (Figs. 5 and 6).

The total length of 12 specimens ranged from 160 to 245 μ , average 205 μ . The distance between the medial

tubercles of appendages II ranged from 32 to 36 μ , average 34 μ . The distance from the tubercles of appendage I to IV varied from 37 to 55 μ , average 44 μ .

Discussion

The protonymph of D. canis has the same general structure as protonymphs of other Demodex species as illustrated by Hirst (1919).

Specimens of the protonymph varied greatly in size. However, no morphological differences were detected between the larger and smaller specimens. The protonymphs shedding deutolarval skins were not morphologically distinguishable from the protonymphs containing forms with adult characteristics.

Sexual dimorphism is not evident in the protonymph nor larvae.

Deutonymph

Description

Dimorphic deutonymphs were not distinguishable from adults except when molting.

Discussion

Hirst (1919) described deutonymphs for several species and in great detail for D. muscardini. His descriptions and figures illustrate the deutonymph as very similar in general structure to the protonymph, yet morphologically distinct.

Sokolovskii (1952) indicated a deutonymphal stage for D. canis but did not adequately illustrate the stage.

I observed specimens with adult characteristics that appeared to be molting (Fig. 22). Herein, such specimens are referred to as male or female deutonymphs. The male penises and the female genital openings were clearly distinguishable, yet a new integument was evident within the opisthosoma. No differences were detected in the size or shape of the distal pedipalpal spines, dorsal pedipalpal spines, chelicerae, legs, and tarsal setae of the molting deutonymphs and adults containing ova.

Hirst (1919, page 32) in reference to D. muscardini stated that besides the protonymphs and deutonymphs:

. . . specimens of another stage are met with which resemble the adults in almost every respect but are more delicate in appearance (apparently being less strongly chitinized); the dorsal spines on the capitulum are poorly developed in these specimens.

Female

The adult and deutonymph females were the largest and most frequently encountered stages of D. canis.

Description

Four pairs of legs appear to have five segments each. The distal segments are quite small and bear two dentate claws each (Figs. 11 and 12).

The membranous gnathosoma contains the pedipalps and

chelicerae. Both styliform chelicerae consist of two members fused distally (Figs. 13 and 14). Each distal pedipalpal plate bears four sharply pointed setae. The basal pedipalpal segment bears a single dorsal seta, slightly less than 4 u in length (Figs. 15 and 16). A horseshoe-shaped structure is present on the posterior ventral surface of the gnathosoma. On either side of this structure a small pore is present (Fig. 11).

The ventral slit-like genital opening is located medial and slightly posterior to coxae IV (Fig. 11).

A small chitin tube is located internally to the posterior one-third of the opisthosoma (Figs. 11 and 17). The tube projects posteriorly from a ventral opening in the integument.

The total length of the specimens ranged from 165 to 285 u. The podosoma varied from 54 to 59 u when measured from the anterior end of the genital slit to the anterior edge of the ventral coxal plate of leg I.

Discussion

The horseshoe-shaped structure and the pores of the gnathosoma were thought by Hirst (1919) to have some respiratory function. The horseshoe-shaped structure is present also on the larvae, nymphs, and males.

The chitin tube in the posterior of the opisthosoma is fragile. Frequently the tube appeared collapsed. However,

gently cleared specimens demonstrated cylindrical tubes. No excretory openings are described for Demodicidae. This chitin tube may function in an excretory system. No such tubes were evident in the larvae, protonymphs, or males.

The length of the opisthosoma is in direct proportion to the distance between the annular striations of the integument. There were approximately 80 annular striations on each opisthosoma of eight specimens. The total length of the eight opisthosomae ranged from 70 to 155 μ . The average distance between striations was obtained by dividing the number of striations per 10 linear μ by 10; averages for the eight specimens ranged from 0.98 to 1.96 μ . The approximate length of each opisthosoma was calculated by multiplying the average distance between striations by 80. The greatest difference of calculated length from the actual length, of the eight opisthosomae measured, was 8 μ .

Male

The male deutonymphs were not distinguished from the adult male, except when molting.

Description

The legs and gnathosoma of the male are similar in structure and size to the female.

The genital opening consists of two semicircular plates located dorsal and medial to coxae I and II (Fig. 18). The

penis has two sclerotized members (Figs. 19 and 20). The dorsal member is pointed distally and is the longer and more narrow. The proximal end connects with the vas deferens. The ventral member serves as a sheath for the distal half of the dorsal member. The retracted penis curves dorsally; when extruded the distal portions are directed posteriorly. Four tubercles are placed dorsally; one above each leg I and II (Fig. 18).

The opisthosoma of the male is more pointed and generally shorter than the female. The total length of the males ranged from 150 to 210 μ . The podosomal length was 53 to 57 μ .

- Fig. 10. Protonymph of Demodex canis, ventral view
- Fig. 11. Female of D. canis, ventral view
- Fig. 12. Distal claw of leg, adult D. canis
- Fig. 13. Chelicerae of adult D. canis, ventral view
- Fig. 14. Left chelicera of adult D. canis
- Fig. 15. Dorsal spine of basal pedipalpal segment, anterior view, adult D. canis
- Fig. 16. Dorsal spine of basal pedipalpal segment, lateral view, adult D. canis
- Fig. 17. Lateral view of internal chitin tube in striated opisthosoma of female D. canis
- Fig. 18. Male of D. canis, dorsal view
- Fig. 19. Male D. canis, sagittal section of podosoma with lateral view
- Fig. 20. Penis of D. canis

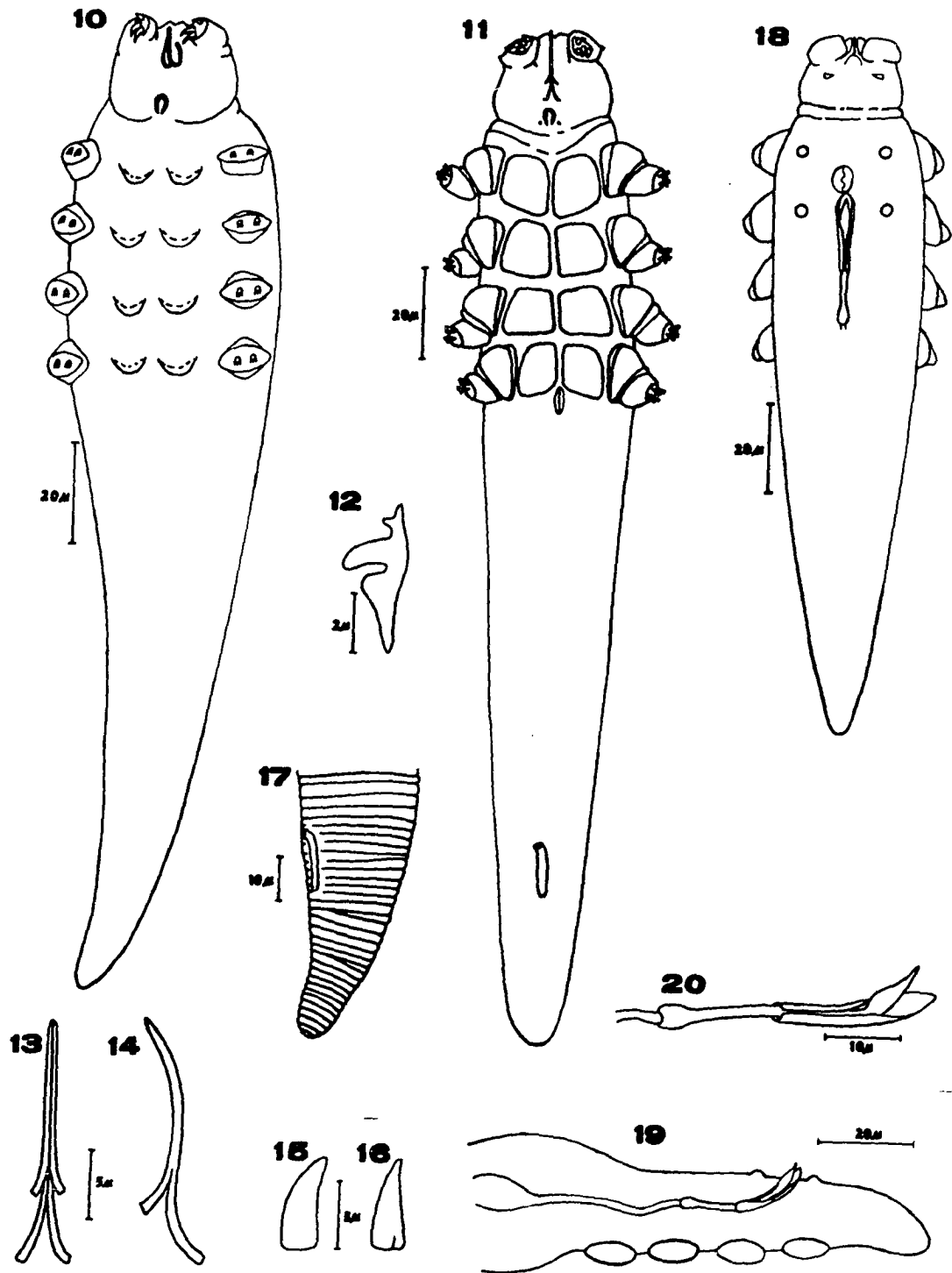


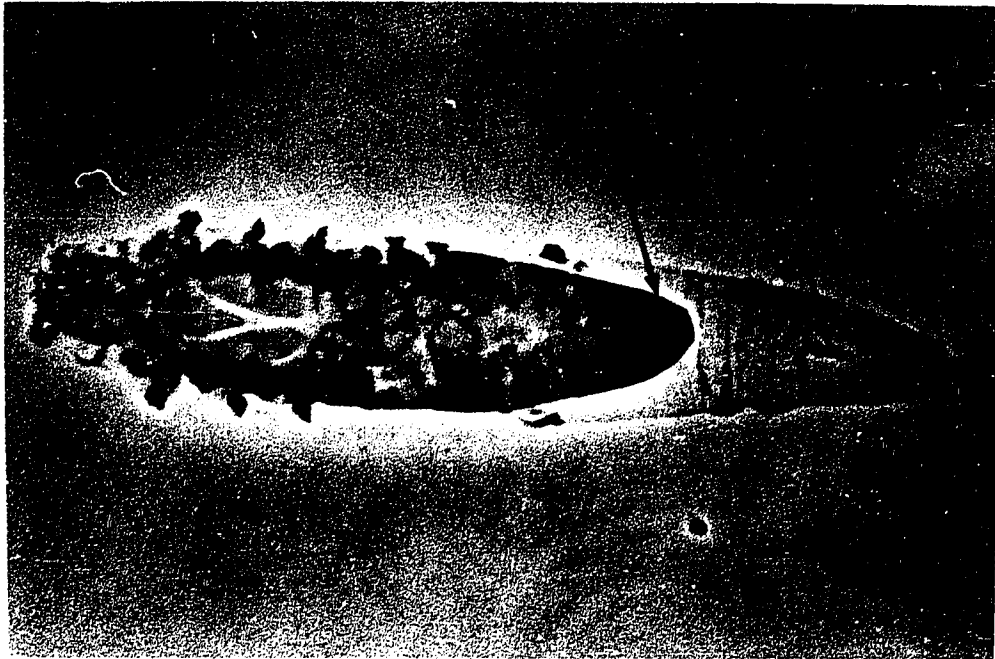
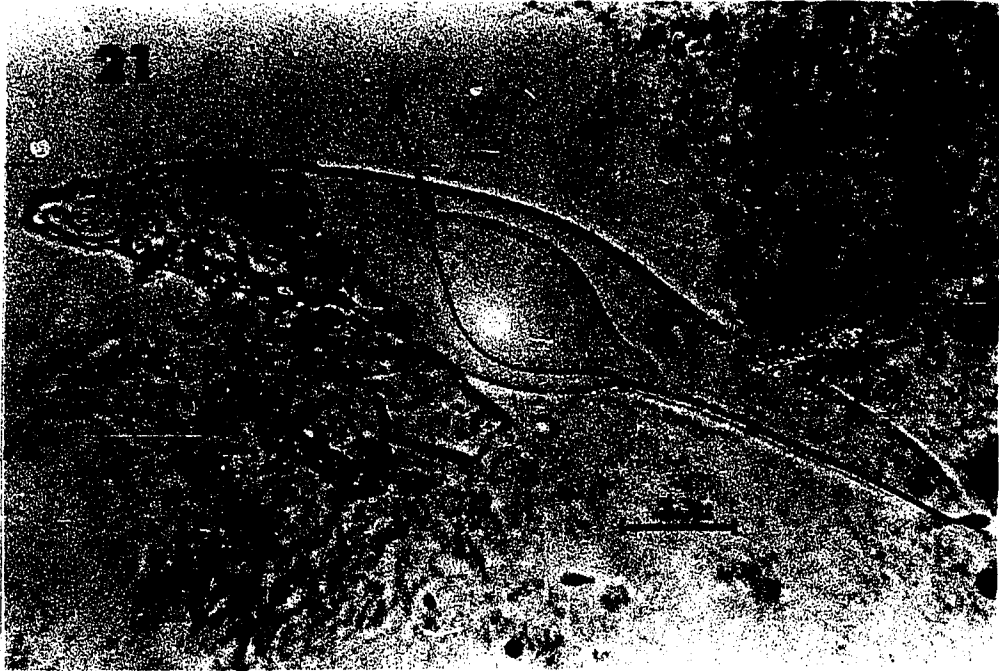
Fig. 21. Ovigerous female of Demodex canis (phase-contrast photomicrograph)

A. Egg shell

Fig. 22. Deutonymph of D. canis before molting, female integument within (phase-contrast photomicrograph)

A. Opisthosoma of female

B. Chitin tube of deutonymph



SUMMARY

The stadia of D. canis are ovum, protolarva, deutolarva, protonymph, deutonymph, and adult. The protolarva has three pairs of non-articulate appendages, each bearing a single tridentate tubercle. The deutolarva has two tridentate tubercles on each of the six non-articulate appendages and a pair of medio-ventral podosomal discs between each pair of appendages. The protonymph has four pairs of medio-ventral discs and four pairs of non-articulate appendages bearing two tridentate tubercles each. The dimorphous deutonymphs were undistinguishable from the adults except when molting. The deutonymphs and adults have four pairs of articulate legs. Each leg bears a pair of dentate claws.

The stadia were described and morphological characters illustrated by line drawings and photomicrographs. Specimens from 75 to 80 per cent ethyl alcohol mounted in a polyvinyl alcohol medium were suitable for study of most characters by phase microscopy.

PART II. DEMODEX CANIS IN INTERNAL TISSUES

INTRODUCTION

The presence of Demodex canis in lymph nodes of dogs with demodectic mange was reported by Cánepa and da Graña (1941). Subsequent publications of de Mello et al. (1943), Unsworth (1946), Enigk (1949), el-Gindy (1952), Gur'ianova and Dulebov (1952), Luckner and Sause (1952) and Koutz (1957) verified the presence of all stages of D. canis in lymph nodes. Various techniques employed by these authors included: (1) direct examination of lymph nodes after maceration and squashing; (2) maceration of tissue in potassium hydroxide, centrifugation, and examination of the sediment; and (3) examination of stained histologic preparations. D. canis was reported most frequently from the mandibular and prescapular lymph nodes. The mites were also reported from nine other lymph nodes. A summary of the lymph nodes reported as infested is presented in Table 1.

Cánepa and da Graña (1945, page 802) reported recovering D. canis in blood extracted from the "vena safena externa." Incisions were made to expose the veins and the blood samples were taken directly from the veins with syringe and needle. The authors felt that this was adequate precaution against contamination of the sample by mites from the skin. Post-mortem blood samples were extracted from the "venas cavas, anterior y posterior." They found one to two Demodex in 10 cc blood samples in 18 of 30 dogs with demodectic mange.

Table 1. Lymph nodes infested by D. canis as reported by seven authors

| Lymph nodes | Authors | | | | | | |
|------------------------|--------------------------------|--------------------|--------------------|-----------------|--------------------|------------------|-----------------|
| | Cánepa (1941) | de Mello (1943) | Unsworth (1946) | Enigk (1949) | el-Gindy (1952) | Lucker (1952) | Koutz (1957) |
| Mandibular | MS ^a H ^b | MS H | MS M ^c | M | M | M | MS M |
| Prescapular | MS | MS H | MS M | M | - | M | MS |
| Medial retropharyngeal | MS | MS H | - | M | - | - | - |
| Iliac | - | - | MS M | M | M | - | MS |
| Axillary | MS | - | - | - | M | - | MS |
| Bronchial | - | - | - | OM ^d | - | - | - |
| Superficial inguinal | MS | MS | - | M | M | - | MS |
| Popliteal | MS | H | - | M | M | - | - |
| Mesenteric | MS | H | - | M | M | - | - |
| Portal | - | - | MS | OM | - | - | - |
| Medistinal | MS | - | - | - | - | - | - |

^aMS = mites found by maceration and smears.

^bH = mites found in histologic preparations.

^cM = mites found in sediment after maceration and centrifugation.

^dOM = no mites in macerated tissue.

Rubin (1957) also reported D. canis in blood samples. Koutz (1957) stated:

In about 20 per cent of the dogs with demodectic mange, mites were found in the blood. In 1 dog, only the larval stages were found; in the others, adult forms as well as nymph and larval stages were found.

After D. canis was reported to be present in the blood vascular and lymphatic systems, several publications appeared reporting the mites in many other tissues and organs of dogs with demodectic mange as summarized in Table 2. Gur'ianova and Dulebov (1952) reported D. canis present in lungs, spleen, tongue muscles, bladder, and intestinal wall. Koutz (1957) published finding the mites in liver, spleen, tongue muscles, kidney, urine, and blood. Also in 1957, Rubin reported D. canis in lungs, liver, and spleen.

The significance of the presence of D. canis in the various internal organs and tissues is not understood. Some researchers (Cánepa and da Graña, 1941; Rathsan and de Paiva, 1943; and Rubin, 1957) supported the theory that a phase of the D. canis life cycle occurs within the lymph nodes. Unsworth (1946) and Enigk (1949) were of the opinion that the presence of the mites in lymph nodes and the circulatory system was accidental or passive and not a necessary phase of the life cycle.

The terminology of Runnells et al. (1960) for cell types and inflammation was used in the following discussion. The observations described were undertaken to determine the location of the mites in the lymph nodes and other organs.

Table 2. Various organs infested by D. canis as reported in six publications

| Organ or tissue | Authors | | | | | |
|-----------------|----------------|----------------|-----------------|-------------------|----------------|--------------|
| | Cánepa (1942) | Cánepa (1945) | Enigk (1949) | Gur'ianova (1952) | Koutz (1957) | Rubin (1957) |
| Lungs | H ^a | - | - | U ^b | - | H |
| Liver | - | - | OM ^c | U | M ^d | H |
| Spleen | H | - | M | U | M | H |
| Tongue muscle | - | - | - | U | M | - |
| Bladder | - | - | - | U | - | - |
| Intestinal wall | - | - | - | U | - | - |
| Blood | - | + ^e | O ^f | - | + | + |
| Urine | - | - | - | - | + | - |

^aH = mites found in histological preparations.

^bU = mites reported, but method undisclosed.

^cOM = no mites found when tissue was macerated.

^dM = mites found by macerating the tissue.

^e+ = mites present.

^fO = no mites found.

METHODS AND PROCEDURES

Tissue samples were taken at necropsy or by biopsy. Care was taken to prevent contamination of the tissue samples with D. canis from the skin. Such contamination could cause false positive results in techniques employing maceration of tissue and examination of the residue for mites. Observation of the mites in situ in histopathologic preparations was considered the only completely reliable method of determining the presence of D. canis in any particular tissue. Unfortunately, histologic preparations are time-consuming.

The most efficient technique for macerating the tissue and examining the residue for D. canis was the following KOH method:

1. Tissue samples placed in 12 ml conical centrifuge tubes;
2. Tubes filled to 10 ml with a 6% potassium hydroxide solution (KOH);
3. Incubated in oven at 75°C until macerated, but no longer than 24 hours;
4. Centrifuged tissue suspension at 500x gravity for at least 3 minutes;
5. Decanted to 0.2 ml with special curved suction pipette as illustrated in Fig. 23;
6. Added approximately 8 ml of "acid chloral hydrate" (40 g chloral hydrate, 25 ml distilled water and

2.5 ml hydrochloric acid);

7. Incubated in oven at 75°C for 4 to 12 hours;
8. Centrifuged at 500x gravity for at least 3 minutes;
9. Pipetted sediment, placed on glass slide and covered with 22 x 40 mm cover glass;

10. Examined with phase microscope at 100 magnification.

Greater concentrations of potassium hydroxide tended to produce an objectionable quantity of crystals in the sediment. Prolonged incubation and temperatures above 90°C damaged ova and larvae. The curved pipette permitted decanting nearly all of the liquid without disturbing the mite-containing sediment. The "acid chloral hydrate" dissolved some tissue left by the hydroxide and reduced the quantity of sediment. The resulting chloral hydrate solution had a refractive index favorable for phase microscopy and identification of mite exoskeletons.

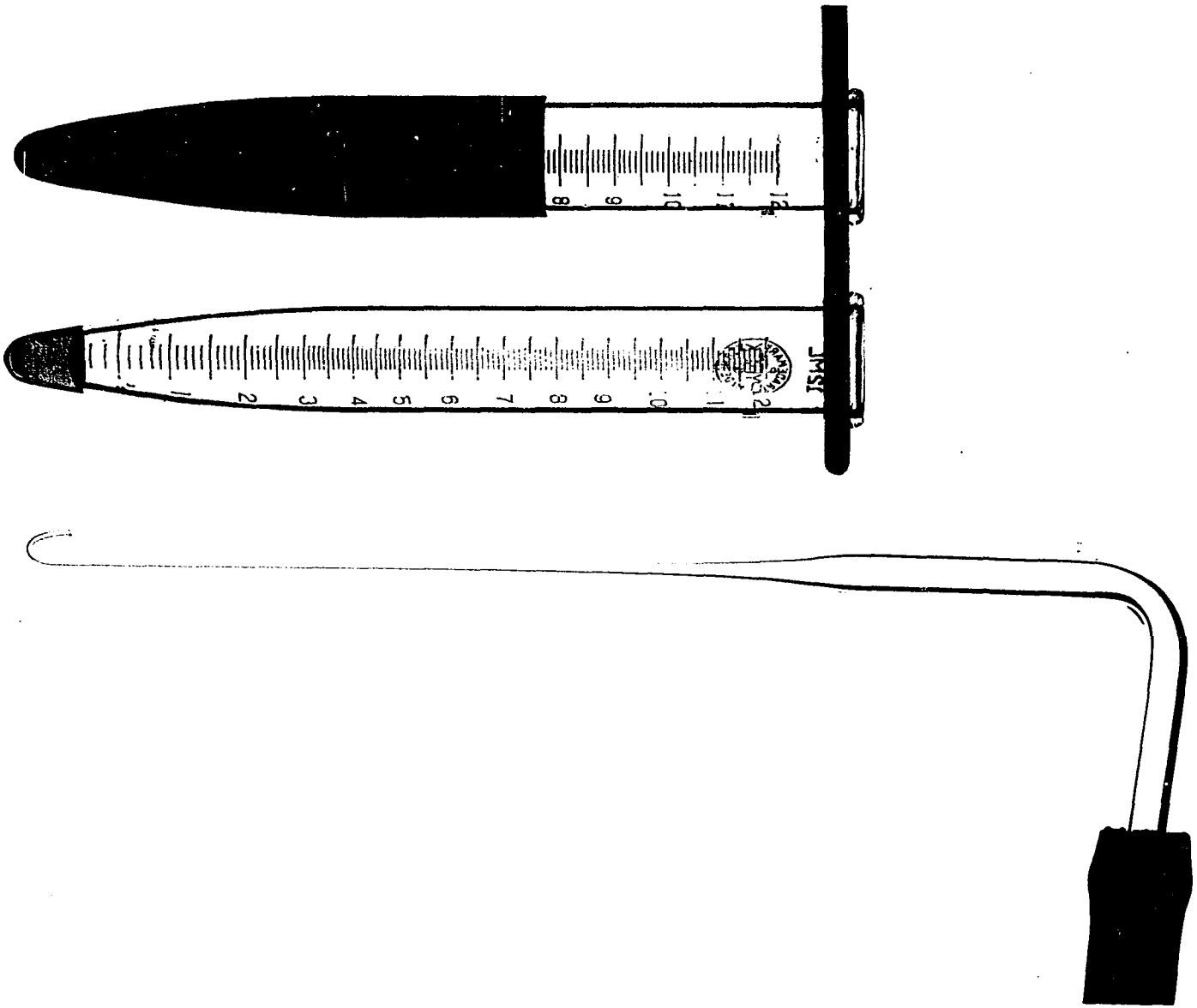
Only portions of some tissue samples were examined by the KOH method. The remainder of each sample was fixed in 4% formaldehyde and preserved in 70% ethyl alcohol. The preserved tissues were embedded in paraffin, cut in sections 10 to 14 μ in thickness, and mounted in series on glass slides. Slides were stained with Mayer's hematoxylin and eosin (see Stain 1., Appendix) using an Autotechnicon¹. The stained

¹The Technicon Co., Chauncey, New York.

Fig. 23. Curved pipette attached to rubber hose from water suction pump. Conical centrifuge tubes (right to left):

Lymph node residue, macerated in 6% potassium hydroxide, after decanting with curved pipette

Residue of lymph node after maceration, decanting, addition of "acid chloral hydrate", and centrifugation



sections were systematically examined by light and/or pseudodark-field microscopy. The pseudodark-field effect was obtained with an A-O phasestar microscope, by using 10X oculars, the 10X medium dark-contrast objective, and the annular diaphragm for the 97X objective. A bright, silver outline of the mite integument located D. canis by the pseudodark-field method (Fig. 24). The mites were located in the tissue sections with light microscopy by searching for the characteristic cluster of tiny blue granules (1 to 2 μ in diameter) of the mite gut as in Fig. 25.

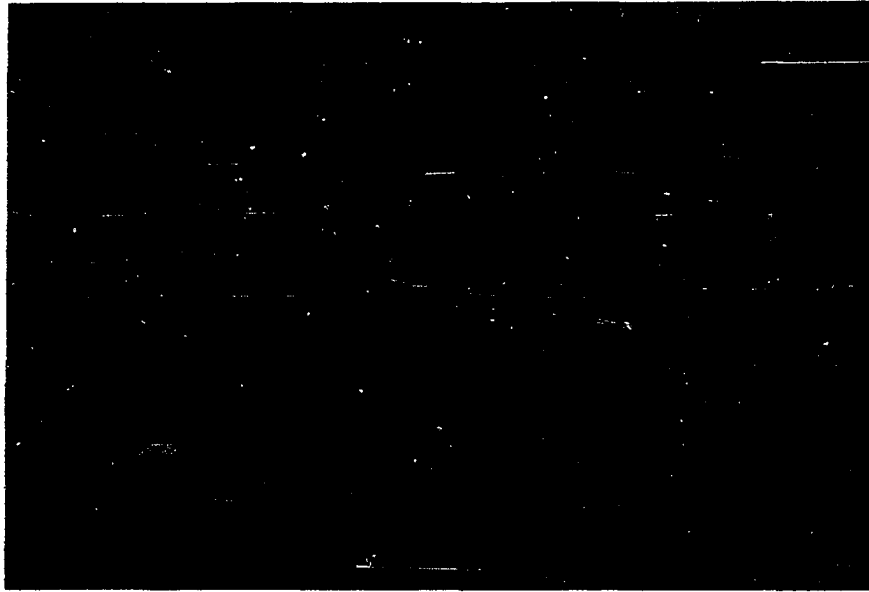


Fig. 24. Pseudodark-field microphotograph of cross section of D. canis in lung. Hematoxylin and eosin. X 200



Fig. 25. Blue granules of D. canis gut, longitudinal section, located in follicle of mandibular lymph node. Hematoxylin and eosin. X 400

OBSERVATIONS AND DISCUSSIONS

Lymph Nodes

The majority of the histopathologic observations were made on tissue samples taken at necropsy from five dogs (8D, 9D, 10D, 11D, and 14D). At death, each dog had a severe demodectic mange. Secondary bacterial invasion of the skin was quite noticeable on 8D and 14D. Each dog had large areas that were nearly devoid of hair. The dog, 10D, was almost without principal (guard) hairs and had very few auxiliary (wool) hairs at death (Fig. 26).

Demodex canis, in all stages, were identified in at least one sample of mandibular, parotid, medial retropharyngeal, iliac, axillary, prescapular, superficial inguinal, and popliteal lymph nodes from the five dogs mentioned above. Table 3 records the presence or absence of D. canis in lymph nodes from the five dogs as determined by the KOH method and histologic preparations as described on pages 32 and 33. The superficial lymph nodes, which receive lymph from large areas of skin, consistently contained specimens of D. canis (Table 3).

As mentioned in the introduction of this section, the fact that D. canis could be found in superficial lymph nodes is well documented. De Mello et al. (1943) published the most complete histopathologic descriptions of lymph nodes containing D. canis. The present histopathologic study was made to

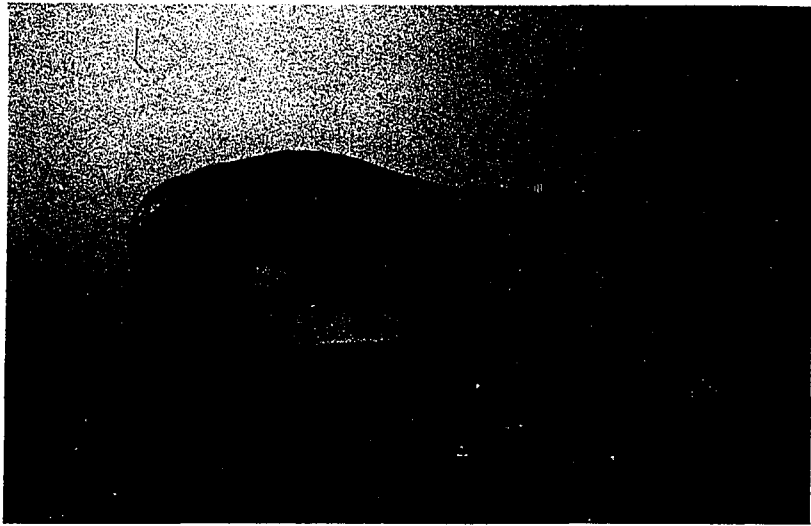


Fig. 26. Dog 10D, almost without hair, two weeks before death

Table 3. Demodex canis in lymph nodes of dogs with severe demodectic mange

| Lymph nodes | Dogs | | | | | | | | | |
|-------------------------|----------------|----------------|----|---|----------------|---|-----|---|-----|---|
| | 8D | | 9D | | 10D | | 11D | | 14D | |
| | K ^a | H ^b | K | H | K | H | K | H | K | H |
| Mandibular | K | H | K | H | O ^c | H | K | H | K | - |
| Parotid | K | H | K | H | O | H | O | H | K | H |
| Medial retro-pharyngeal | - | - | O | H | K | H | K | O | K | H |
| Iliac | K | H | K | O | K | H | O | O | - | - |
| Prescapular | K | H | K | H | K | H | - | - | K | H |
| Axillary | - | - | K | H | K | H | - | - | K | H |
| Superficial inguinal | K | H | O | - | K | H | K | H | K | H |
| Popliteal | - | - | K | H | K | - | - | - | K | H |

^aK = mites found by KOH method.

^bH = mites found in histologic preparations.

^cO = no mites.

determine the exact locations of the mites and observe the reaction of cells surrounding the mites.

The vast majority of mites observed were located in peripheral (cortical) sinuses immediately medial or below the connective tissue of the capsule. Frequently, D. canis was observed within lymph ducts and capillaries in or just outside the capsule. Rarely, a mite was seen in a primary lymphocyte follicle or in a lymph capillary located in a

trabecula of the medullar region. No mites were observed within the medullar sinuses.

Foreign-body giant cells (polynucleated macrophages) were present in all Demodex-containing lymph nodes examined histologically. Runnells et al. (1960, page 203) stated, ". . . when great phagocytic power is needed, the macrophages fuse together and form foreign-body giant cells." Observations indicated that giant cells were formed in response to the presence of the mites. A survey was conducted by selecting stained slides, from the series of each lymph node, at great enough interval to prevent duplication in recording the presence of giant cells and mites. On each selected slide, a section near the middle of the series was surveyed. Close associations of giant cells and D. canis were recorded (Fig. 27). If a mite alone was found in the surveyed section, the preceding and subsequent sections were examined to make certain a giant cell was not associated with this mite in other sections. The same tracking procedure was done on any solitary giant cell found in the original survey section.

On the 66 slides that were surveyed, 180 giant cell-D. canis relationships were noted; 59 mites were observed with no apparent association with a giant cell. Some of the solitary mites may have recently arrived in the lymph node and sufficient time may not have lapsed for giant cell formation. There were 27 giant cells which had no apparent association with a mite. Some, perhaps, had already removed the foreign

body. The giant cells were observed only in the peripheral sinuses and lymph ducts (Figs. 28 and 29). No giant cell was observed in the medullar region of any lymph node. The distribution of the giant cells coincided with the areas in which D. canis was noted. Giant cells were not observed in three lymph nodes which did not contain D. canis; although, other nodes from the dogs 9D and 11D were invaded by D. canis and giant cells were present.

Runnells et al. (1960) discussed several canine diseases (tuberculosis, actinobacillosis, actinomycosis, staphylococcosis, blastomycosis, coccidioidomycosis, histoplasmosis, and mucormycosis) in which the infectious organisms can stimulate giant cell formation in lymph nodes. Because giant cells were not observed in lymph nodes free of Demodex, it was felt that there was no disease, other than demodectic mange, causing the formation of giant cells in the lymph nodes of my experimental animals.

In addition to the mites and their accompanying giant cells, the following reactions were noted: macrophage proliferation, connective tissue infiltration, and lymphatic thrombosis. The areas of macrophage proliferation were located, primarily, in the peripheral sinuses. An increase of connective tissue accompanied the macrophage proliferation in some instances as in Fig. 30. The macrophages were often arranged in whorls as in Fig. 29, and were characteristic of lymph nodes with moderate to numerous D. canis present.

Fig. 27. D. canis (arrow) in giant cell in parotid lymph node. Hematoxylin and eosin. X 926

Fig. 28. Macrophage proliferation in peripheral sinus of mandibular lymph node. Hematoxylin and eosin. X 93

Fig. 29. Macrophage whorl in prescapular lymph node. Hematoxylin and eosin. X 400



Occasionally a mass of macrophages and connective tissue cells was observed projecting from the cortex into the lumen of a lymph duct. Fig. 31 illustrates such a mass consisting almost entirely of macrophages and mites.

The cellular reaction in lymph nodes containing D. canis was chronic inflammation. The macrophage proliferation can be classified as hyperplastic inflammation. Lymph node reactions with D. canis were diagnosed by de Mello et al. (1943, page 173) as "linfogranuloma com parasita." Runnells et al. (1960, page 704) stated: "The mite is carried to the regional lymph nodes by the lymphatics and produces a chronic serous lymphadenitis."

Organized exudates or parasitic lymphatic thrombi were observed in some of the lymph ducts of five dogs (8D, 9D, 14D, 38D, and 71D). All of these dogs had severe chronic demodectic mange. The lymph ducts containing thrombi were accompanying prescapular, mandibular, parotid, axillary, or popliteal lymph nodes. All of the eight thrombi observed appeared spongy or loose in consistency and the ducts were not completely occluded. The constituents of the thrombi varied and included D. canis, lymphocytes, macrophages, giant cells, neutrophils, erythrocytes, protein deposits (hyaline masses), and fibrin. Lymphocytes and protein deposits were the major constituents. The protein deposits stained deep pink or red with hematoxylin and eosin (Fig. 32). Phosphotungstic acid hematoxylin (PTAH) stained the protein deposits



Fig. 30. Connective tissue (green) and macrophages in peripheral sinus of parotid lymph node. Gomori's one step trichrome. X 200



Fig. 31. Mass of macrophages and connective tissue in lymph duct of mandibular lymph node. Hematoxylin and eosin. X 200

pale to dark blue. Fibrin was observed in small quantities. With PTAH reaction, a few strands of fibrin stained dark blue.

Figs. 32, 33, and 34 are of the same section of a lymphatic thrombus associated with a mandibular lymph node. The section was stained first with hematoxylin and eosin then destained with acid alcohol. The section was subsequently stained (then destained with acid alcohol) with Weigert's fibrin stain and phosphotungstic acid hematoxylin. These staining procedures are outlined as Stains 1, 2, and 3 in the Appendix. Figs. 35, 36, and 37 were taken of consecutive sections stained by hematoxylin and eosin, PTAH, and Gomori's one step trichrome. The last staining procedure is outlined as Stain 5 in the Appendix.

A thrombus, taken by biopsy in connective tissue of a prescapular lymph node from dog 71D, was well organized (Fig. 38). Blood capillaries were present among the macrophages, mites, and connective tissue cells. A thrombus was also observed in a lymph duct in the connective tissue associated with a parotid salivary gland. Fibrin strands were demonstrated in this thrombus by the PTAH reaction (Figs. 39 and 40).

De Mello et al. (1943) mentioned micro-abscesses in lymph nodes from dogs with demodectic mange and believed the reactions were the result of secondary bacterial infections. I observed small neutrophil concentrations in the periphery of

Fig. 32. Lymphatic thrombus associated with a mandibular lymph node. Hematoxylin and eosin. X 200

Fig. 33. Same section of lymphatic thrombus as in Fig. 32, after destaining with acid alcohol and staining with Weigert's fibrin stain. X 200

Fig. 34. Same section of lymphatic thrombus as in Fig. 33, after destaining with acid alcohol and staining with phosphotungstic acid hematoxylin. X 200

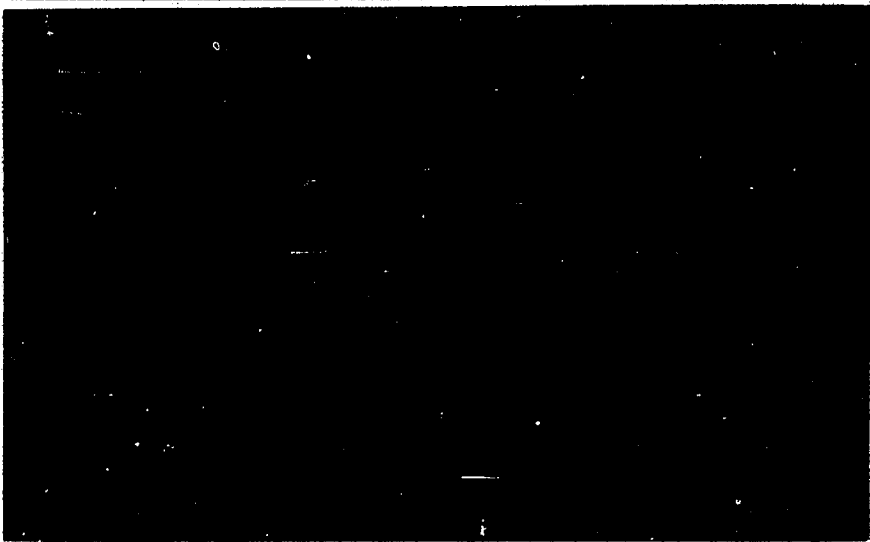
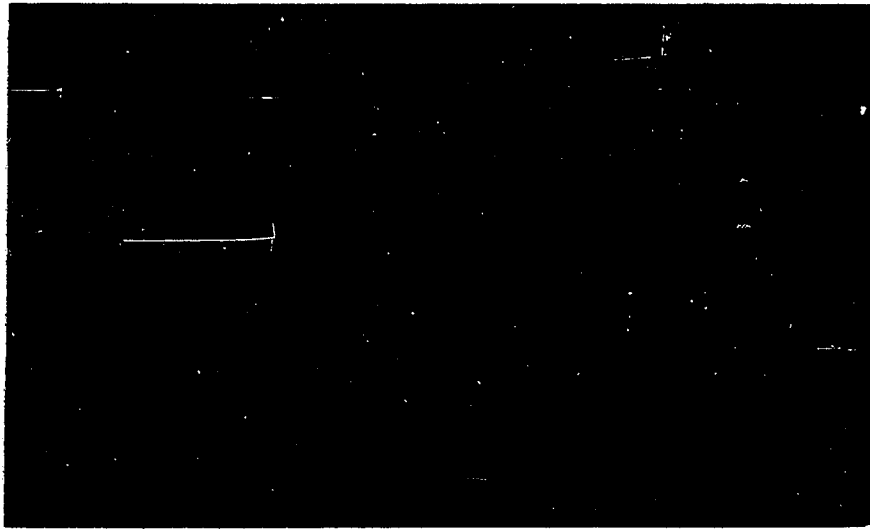


Fig. 35. Lymphatic thrombus associated with an axillary lymph node. Hematoxylin and eosin. X 400

Fig. 36. Lymphatic thrombus, section adjacent to that in Fig. 35. Phosphotungstic acid hematoxylin. X 400

Fig. 37. Lymphatic thrombus, section adjacent to that in Fig. 36. Gomori's one step trichrome. X 400

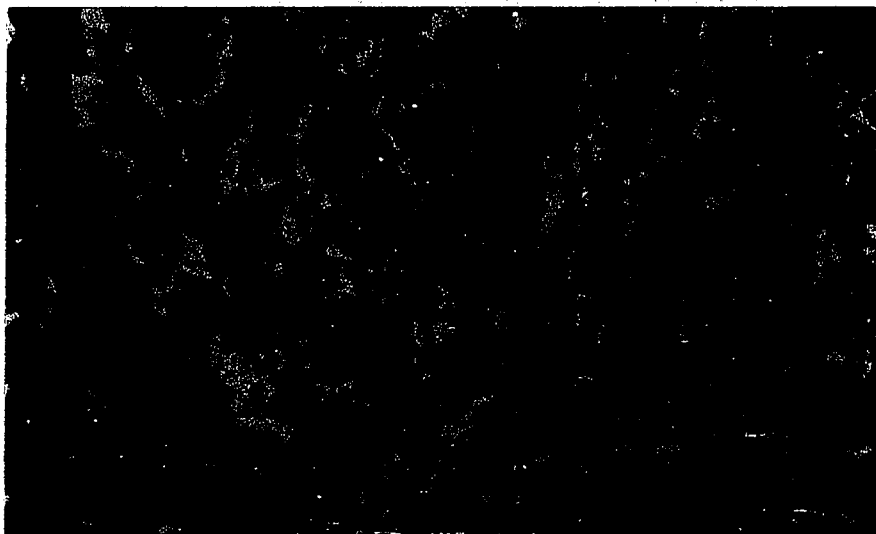
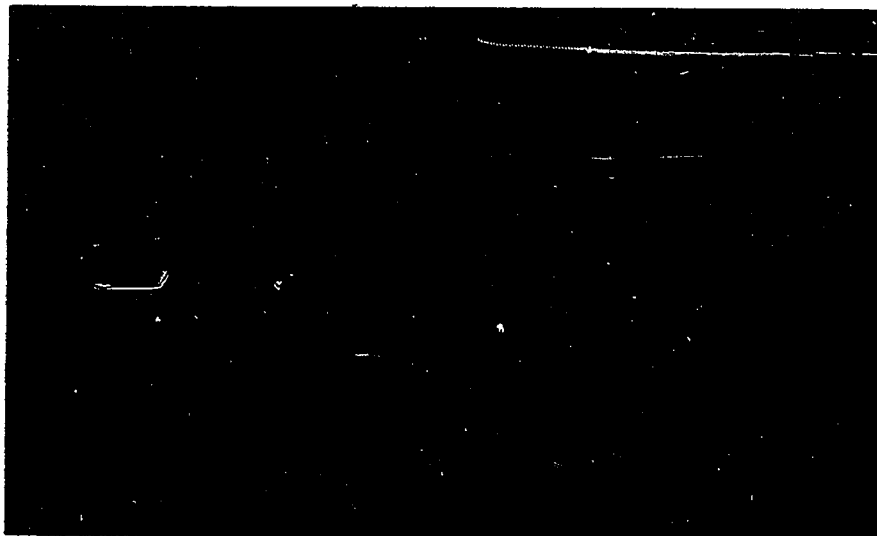




Fig. 38. Organized thrombus in lymph duct located in peripheral connective tissue of prescapular lymph node. Gomori's one step trichrome. X 200

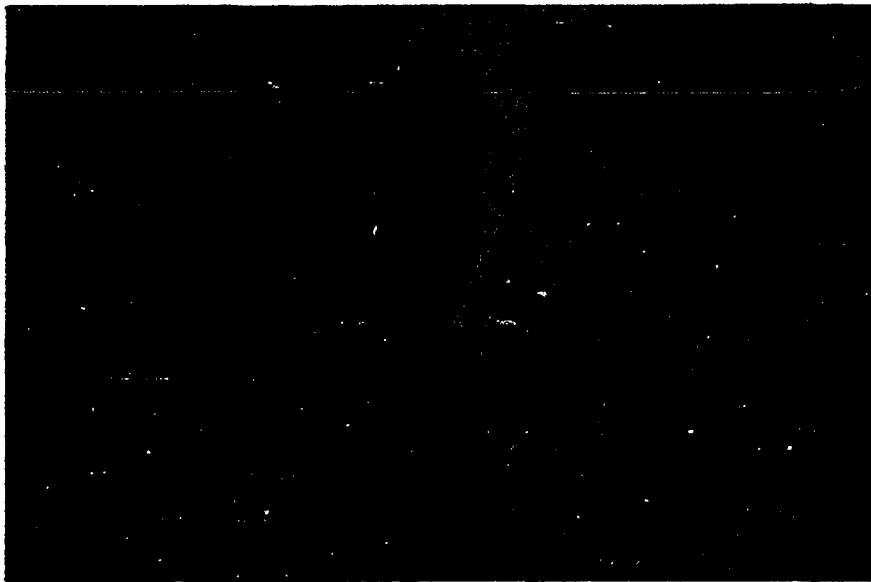


Fig. 39. Thrombus in lymph duct located in peripheral connective tissue of parotid salivary gland. Phosphotungstic acid hematoxylin. X 93

only two prescapular lymph nodes from 71D and 54D. The dogs had a severe bacterial infection in the skin. Erythrocytes and neutrophils were abundant in an afferent duct of the prescapular lymph node of 71D, which also had an organized thrombus.

The relationship of skin lesions, and the presence or absence of D. canis in superficial lymph nodes, is not clear. The manner by which the mites gain entry into the blood and lymph vessels has not been demonstrated. It is possible that during extensive inflammation of the dermis, mites leave necrotic hair follicles and enter dilated blood and lymph capillaries. Some of the mites then enter the superficial lymph nodes via the afferent lymph ducts from the skin. It is doubtful that a mite entering a lymph node can escape the entangling reticulo-endothelium (Fig. 41) and phagocytosis by macrophages and giant cells.

Contrary to reports of some authors, I have never found D. canis in lymph nodes from dogs without visible skin lesions. Cánepa and da Graña (1945) reported finding mites in macerated mandibular lymph nodes from two pups, aged one month, but no mites were found in several skin scrapings from the head. From one of the pups, they reported recovering two mites in 2.5 cc of blood extracted from the posterior vena cava. One month later (aged two months), litter-mates of the two pups were positive for Demodex, but no skin lesions were noticed.

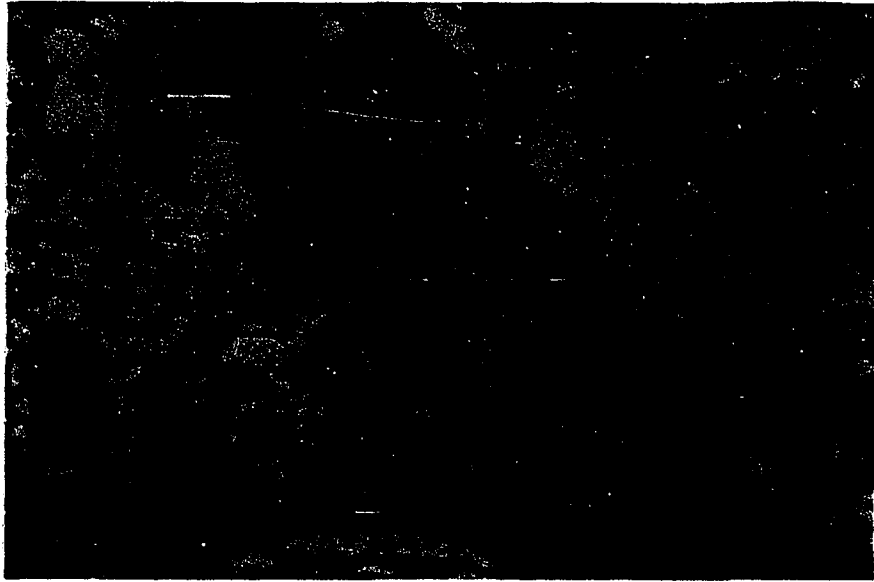


Fig. 40. Lymphatic thrombus in peripheral connective tissue of parotid salivary gland, same section as in Fig. 39. Phosphotungstic acid hematoxylin and eosin. X 400

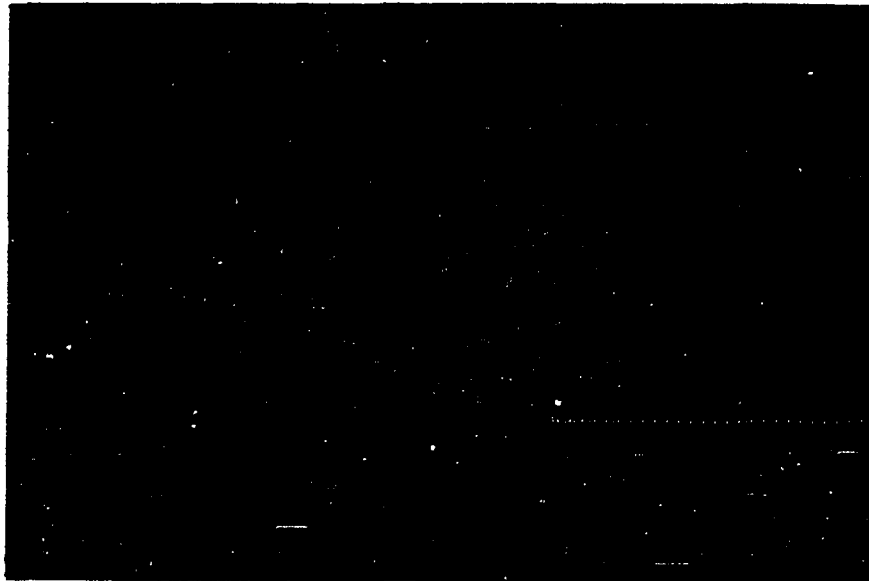


Fig. 41. Prescapular lymph node stained by silver impregnation for reticulin. Cross section of D. canis (arrow) in sinus. X 93

Watson (1948, page 349), while discussing D. canis, reported: "I have found the parasite present in the submaxillary lymph glands of newly born pups and in pups to the age of three months which have never shown clinical signs." Watson did not state methods used in examining these lymph nodes. Koutz (1957) reported mites in the residue of dissolved tongue muscle, mandibular and prescapular lymph nodes, but not in the eyelids or skin samples from one dog.

Table 4 presents observations on the condition of lymph nodes and skin of 16 dogs. No mites were found by the KOH method in lymph nodes of ten dogs, ranging in age from 2.5 days to 3 years, that had mites in their skin but no clinical symptoms. Skin lesions were visible on three dogs, but no mites were found in the lymph nodes (Figs. 42 and 43). In one dog, 38D, 30 mites were found in the left prescapular node taken by biopsy (Figs. 44 and 45). One month later, as the skin began to recover, one mite was found in the right prescapular node. Two dogs, 12D and 7D, had skin lesions for 16 and 18 months and the prescapular lymph nodes were negative 8 and 10 months after the skin lesions were no longer visible. No mites were recovered from mandibular and prescapular lymph nodes taken by biopsy from 19D and 37D. A chronic demodectic mange was evident on 19D for nine months preceding the biopsy and six months for 37D. The observations on lymph nodes from 16 dogs, Table 4, indicate that D. canis is not likely to be present in lymph nodes of dogs

Table 4. Presence of D. canis in lymph nodes in relation to skin condition

| Dog No. | Skin condition ^a and age at lymph node examination | Lymph node(s) | History of skin condition |
|---------|--|---|--|
| 7D | no visible lesions, 3 years | prescapular negative (biopsy) | chronic demodectic mange for 18 months ended about 10 months before biopsy |
| 12D | no visible lesions, forehead skin biopsy negative, 2 years | prescapular negative (biopsy) | chronic demodectic mange for 16 months ended about 8 months before biopsy |
| 15D | no visible lesions, a few mites present in skin of lower jaw, 1 year | mandibular, parotid, prescapular, axillary, medial retropharyngeal; all negative (necropsy) | lesions on forehead at 2 months of age, lasted for 2 months, disappeared about 8 months before necropsy |
| 18D | lesions visible on lower jaw and forelegs, 9 months | mandibular, parotid, medial retropharyngeal, superficial inguinal, prescapular, axillary, popliteal, iliac; all negative (necropsy) | litter-mate of 15D, mites present in skin of forehead at 2 months of age, visible lesions began at 7½ months |
| 19D | visible lesions on back, Fig. 42, 15 months | mandibular and pre-scaphular negative (biopsy) | litter-mate of 15D, chronic demodectic mange from 3 months of age to biopsy at 14 months |
| 21D | a few mites in forehead, no visible lesions, 2½ days | axillary and mandibular negative (necropsy) | |

^aMites were present in the skin of all dogs listed.

Table 4 (Continued)

| Dog No. | Skin condition ^a and age at lymph node examination | Lymph node(s) | History of skin condition |
|---------|--|---|---|
| 22D | no visible lesions, few mites in forehead, 9 months | mandibular and pre-scapular negative (necropsy) | litter-mate of 21D, mites found by skin scrapings at age 2 months, very few mites at necropsy |
| 41D | no visible lesions, few mites in neck, 3 months | mandibular, popliteal, prescapular, all negative (necropsy) | a few mites found in skin scraping 1 month before necropsy |
| 42D | no visible lesions, but a few mites in neck, 3 months | mandibular, popliteal, prescapular, all negative (necropsy) | litter-mate of 41D, a few mites in skin scraping 1 month before necropsy |
| 48D | no visible lesions, but mites in neck, 3½ months | mandibular, popliteal, prescapular, all negative (necropsy) | skin biopsy from head negative 1 month before necropsy |
| 49D | no visible lesions, but mites in skin of flank, 3 months | mandibular, popliteal, prescapular, all negative (necropsy) | litter-mate of 48D, skin biopsy from head was negative 1 month before necropsy |
| 58D | no visible skin lesions, mite in forehead and shoulder, 3½ weeks | mandibular negative (necropsy) | |
| 37D | lesions on back, Fig. 43, 8 months | mandibular and pre-scapular negative (biopsy) | visible skin lesions for 6 months |

Table 4 (Continued)

| Dog No. | Skin condition ^a and age at lymph node examination | Lymph node(s) | History of skin condition |
|---------|--|--|---|
| 38D | visible lesions on back at 7 months (Fig. 44) and 8 months (Fig. 45) | 30 mites in prescapular (biopsy), 1 month later 1 mite in prescapular (biopsy) | litter-mate of 37D, visible lesions for 6 months, skin recovering at 2nd biopsy |
| 54D | extensive skin lesions, bacterial infection, 1 year | prescapular positive (biopsy) | visible skin lesions 2 months before biopsy |
| 71D | extensive skin lesions, bacterial infection, 1 year | prescapular positive (biopsy) | extensive skin lesions 2 months before biopsy |

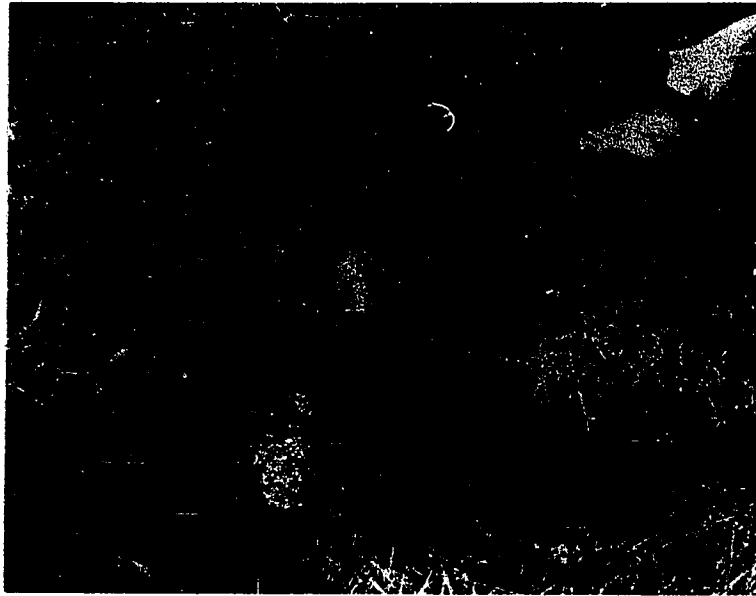


Fig. 42. Dog 19D, 15 months old, with demodectic mange for 12 months. No mites found in mandibular and pre-scapular lymph nodes at biopsy

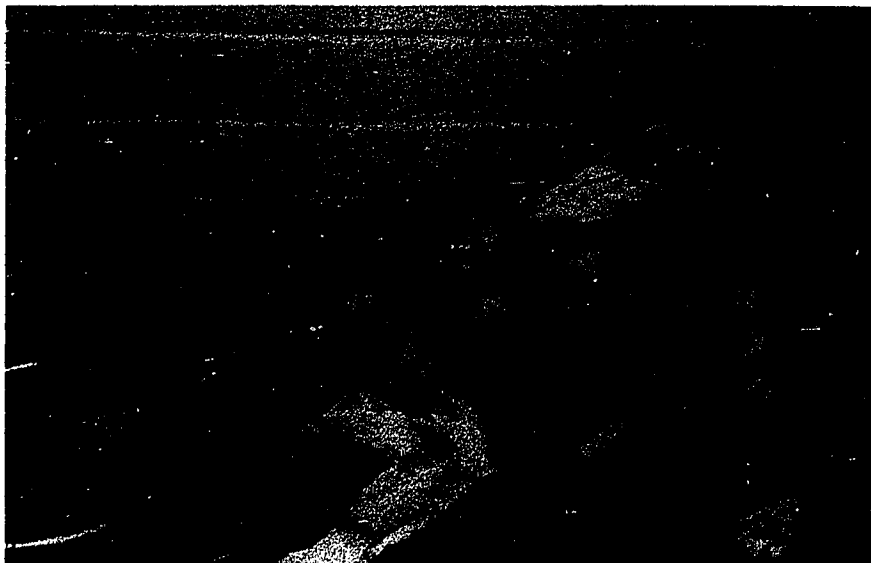


Fig. 43. Dog 37D with demodectic mange for six months. No mites found in mandibular and prescapular lymph nodes at biopsy

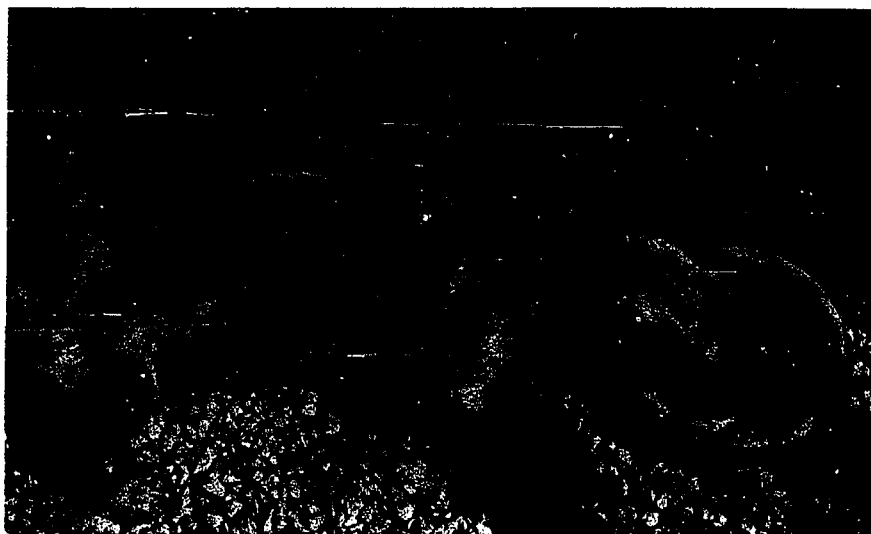


Fig. 44. Dog 38D with demodectic mange for six months; 30 mites recovered from left prescapular lymph node at biopsy

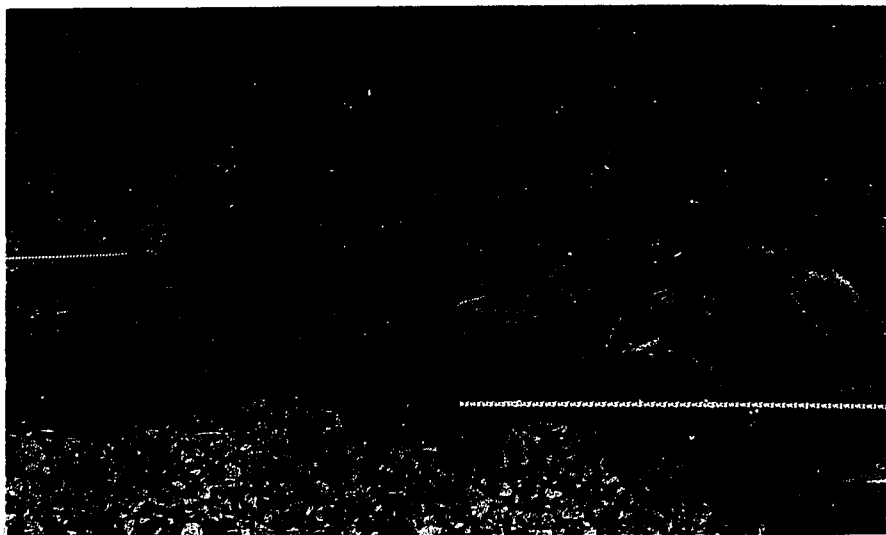


Fig. 45. Dog 38D with demodectic mange for seven months; one mite recovered from right prescapular lymph node at biopsy

without extensive skin lesions.

A portion of axillary lymph node of 14D was examined by the KOH method. A total of 160 mites was observed; 47 larvae, 34 nymphs, and 79 adults. The count is presented in Table 5. No ova were observed. The last nymphal stage was impossible to distinguish from the adult. The ratio, larvae: nymphs:adults, found in this particular lymph node was similar to some differential counts made on skin samples; however, the ratio varied greatly in different areas of skin on the same dog. Not all skin samples have such a high percentage of immature forms.

Table 5. Stages of D. canis observed in residue of an axillary lymph node

| | Proto-larvae | Deuto-larvae | Larvae undiscerned | Proto-nymphs | Fe-males | Males | Adults undiscerned |
|-----------------|--------------|--------------|--------------------|--------------|-----------|------------------------|--------------------|
| Number of mites | <u>23</u> | <u>12</u> | <u>12</u> | <u>34</u> | <u>40</u> | <u>12</u> | <u>27</u> |
| Total | Larvae 47 | | | Nymphs 34 | | Adults 79 ^a | |

^aDeutonymphs not differentiated from adults.

Salivary Glands

The KOH method indicated that D. canis was present in mandibular and parotid salivary glands from the dogs 8D, 9D, 11D, and 14D. These same dogs had many mites in their super-

ficial lymph nodes. In histologic preparations, D. canis was observed only in lymph vessels associated with the salivary glands. The lymph ducts were located in the capsular and interlobular connective tissues of the salivary glands. No mites were observed in the secretory portion of the glands. D. canis was observed in histologic preparations of mandibular and parotid salivary glands from 8D. Mites were also observed in parotid salivary glands of 9D and 14D. There were few mites; the maximum was three individuals per gland.

Fig. 46 shows D. canis in a large lymph duct surrounded by peripheral connective tissue of a parotid salivary gland. A lymphatic thrombus was also present in this lymph duct. A mite was observed in a small lymph duct in the connective tissue of a mandibular salivary gland, Fig. 47.

Thyroid

In a histologic preparation of thyroid from 11D, one D. canis was located in a sinus between the capsule and the follicles of the thyroid gland (Fig. 48). A mite was observed also in the capsular connective tissue of the same thyroid (Fig. 49). The KOH method indicated D. canis was present also in tissue samples of thyroid from the dogs, 10D and 14D. However, no mites were observed in the histologic preparations of thyroid from 10D and 14D.



Fig. 46. Thrombus with D. canis (arrow) in lymph duct located in peripheral connective tissue of parotid salivary gland. Hematoxylin and eosin. X 200



Fig. 47. Cross section of D. canis in lymph duct in connective tissue of mandibular salivary gland. Hematoxylin and eosin. X 200

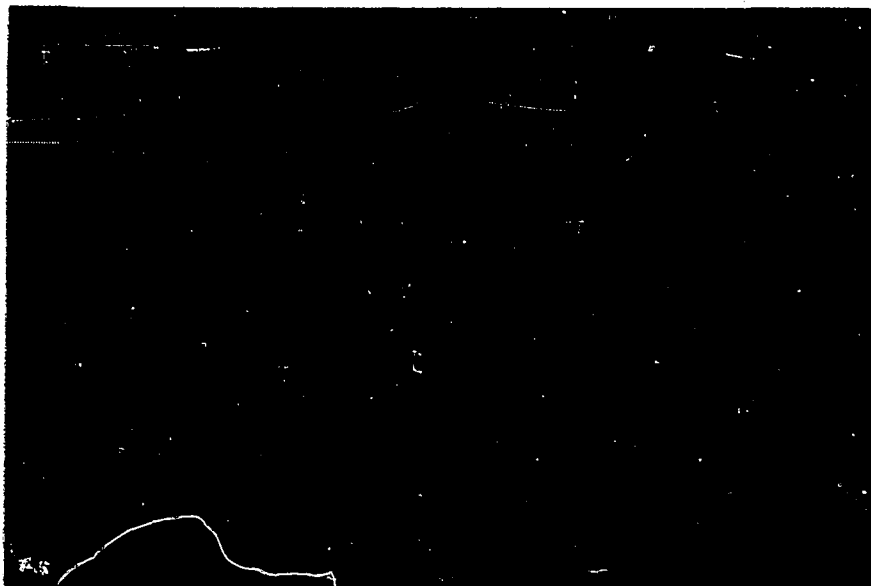


Fig. 48. Thyroid with cross section of D. canis in sinus between capsule and follicle. Hematoxylin and eosin. X 400



Fig. 49. Thyroid with cross section of D. canis in capsular connective tissue. Hematoxylin and eosin. X 400

Lungs

D. canis was observed in histologic preparations of lung tissue from the dogs 8D, 9D, and 14D. Some of the mites apparently were lying free in alveoli at fixation of the tissue as in Fig. 50. Other mites were closely associated with aggregates of macrophages as in Fig. 51. Cănepa and da Graña (1942) published a photomicrograph showing a mite in a section of lung.

Histologic preparations of bronchial lymph nodes from 10D and 14D were negative for D. canis. However, one mite was recovered, by the KOH method, from a bronchial lymph node of 14D.

Other Organs and Tissues

Table 6 summarizes various other organs examined by the KOH method. As noted in Table 2, other authors have reported mites from liver, spleen, tongue muscle, bladder, and intestinal wall. I found a few mites by the KOH method in liver samples from 11D and 14D. However, no mites were observed in histologic preparations.



Fig. 50. Cross section of D. canis in lung alveolus.
Hematoxylin and eosin. X 400

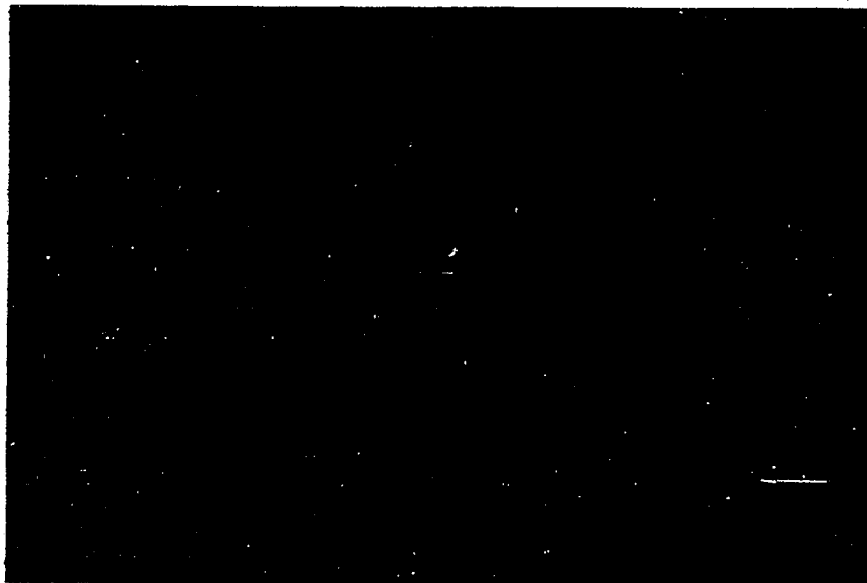


Fig. 51. Macrophages associated with D. canis in lung.
Hematoxylin and eosin. X 400

Table 6. Location of D. canis recovered from five dogs with severe demodectic mange

| Tissue sample | Dog | | | | | | | | | |
|------------------------|----------------|----------------|----|---|-----|---|----------------|---|----------------|---|
| | 8D | | 9D | | 10D | | 11D | | 14D | |
| | K ^a | H ^b | K | H | K | H | K | H | K | H |
| Lymph nodes: | | | | | | | | | | |
| Mandibular | + | + | + | + | 0 | + | + | + | + | - |
| Parotid | + | + | + | + | 0 | + | 0 | + | + | + |
| Medial retropharyngeal | - | - | 0 | + | + | + | + | 0 | + | + |
| Iliac | + | + | + | 0 | + | + | 0 | 0 | - | - |
| Prescapular | + | + | + | + | + | + | - | - | + | + |
| Axillary | - | - | + | + | + | + | - | - | + | + |
| Superficial inguinal | + | + | 0 | - | + | + | + | + | + | + |
| Popliteal | - | - | + | + | + | + | - | - | + | + |
| Mesenteric | - | - | - | - | 0 | - | - | - | - | - |
| Bronchial | - | - | - | - | 0 | 0 | - | - | + ^c | 0 |
| Ileocolic | 0 | - | - | - | 0 | - | - | - | - | - |
| Renal | - | - | - | - | - | - | - | - | 0 | - |
| Other organs: | | | | | | | | | | |
| Tonsil | + | 0 | 0 | 0 | 0 | 0 | - | - | + ^c | 0 |
| Thymus | - | - | - | - | + | 0 | - | - | - | - |
| Thyroid | - | - | - | - | + | 0 | 0 | + | + ^d | 0 |
| Lung | + | + | + | + | + | 0 | 0 | 0 | + | + |
| Liver | 0 | - | 0 | - | 0 | - | + ^e | - | + ^e | 0 |
| Adrenal | - | - | 0 | - | 0 | - | 0 | - | - | - |
| Kidney | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| Pancreas | - | - | 0 | - | 0 | - | - | - | 0 | - |
| Spleen | - | - | 0 | - | 0 | - | 0 | - | 0 | - |
| Brain | 0 | - | 0 | - | 0 | - | - | - | - | - |
| Heart muscle | - | - | 0 | - | - | - | - | - | - | - |
| Bladder | - | - | 0 | - | - | - | - | - | - | - |
| Tongue muscle | - | - | 0 | - | - | - | - | - | - | - |
| Parotid salivary | + | + | 0 | + | 0 | 0 | - | - | + | + |
| Mandibular salivary | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Intestine | 0 | - | - | - | - | - | 0 | - | - | - |
| Stomach | 0 | - | - | - | - | - | - | - | - | - |

^aK = KOH method.^bH = Histologic preparations.^c1 mite.^d4 mites.^e3 mites.

SUMMARY

D. canis was observed in mandibular, parotid, medial retropharyngeal, iliac, prescapular, axillary, superficial inguinal, and popliteal lymph nodes. The mites were primarily located in the peripheral sinuses and lymph ducts associated with the lymph nodes. The reaction of the lymph nodes to the mites was chronic inflammation. Foreign-body giant cells were associated with the mites. Areas of macrophage proliferation were prominent in the periphery. An increase in connective tissue cells was noted in the periphery of the infected lymph nodes. Thrombosis was noted in lymph vessels accompanying some prescapular, mandibular, parotid, and axillary lymph nodes.

A total of nine lymphatic thrombi were observed in five dogs with demodectic mange. The thrombi were spongy or loose in consistency and the ducts were not completely occluded. The constituents of the thrombi included D. canis, macrophages, giant cells, neutrophils, lymphocytes, erythrocytes, protein deposits, and fibrin. A thrombus was observed in a lymph duct in the peripheral connective tissue of a parotid salivary gland.

D. canis were recovered from lymph nodes of dogs with severe chronic demodectic mange. No mites were found in lymph nodes of ten dogs with small populations of D. canis, but with few or no visible skin lesions.

Of the 160 mites recovered from a portion of an axillary lymph node, 47 were larvae, 34 nymphs, and 79 adults. A similar ratio of larvae:nymphs:adults was observed in some samples of skin.

D. canis were observed in lymph vessels in the connective tissue of one mandibular and two parotid salivary glands. The glands were taken from three dogs. Thrombosis was observed in one such lymph vessel. The mites were few and no mites were observed in the secretory portions of the salivary glands.

Mites were observed in the thyroid from one dog. One mite was in a sinus between the connective tissue of the capsule and a follicle of the thyroid. An additional mite was located in the connective tissue of the capsule.

D. canis were observed in the lungs of three dogs. Some of the mites were free in alveoli. Others were closely associated with aggregates of macrophages.

The significance of D. canis in blood, lymph vessels, lymph nodes, lungs, and other organs is not understood. It appears that the mites gain entrance to blood and lymph vessels from necrotic hair follicles. Once the mites enter these vessels they are transported and/or crawl to various locations in the host's body. The reaction of the lymph nodes to the mites makes one doubt that multiplication of the mites occurs here. They probably never escape the lymph nodes.

PART III. TRANSMISSION OF DEMODEX CANIS

INTRODUCTION

Several theories have been proposed concerning the transmission of D. canis from one dog to another. Cánepa and da Graña (1945) were the leading proponents of peroral transmission. Prenatal transmission (in utero) was suggested by Kirk (1950) and Rubin (1957). Unsworth (1946) and Enigk (1949) presented data from experiments designed to demonstrate transmission of D. canis by direct application of mites to canine skin.

The difficulty of accurately evaluating the success or failure of transmission attempts has plagued all researchers on this problem, for successful transmission rarely produces visible skin lesions. Often careful examination of large areas of skin is necessary to detect small populations of D. canis.

METHODS AND MATERIALS

Puppies, to be reared by hand, were placed in individual compartments of incubators as in Fig. 52. Each compartment was 1 square foot in area. Heat was provided by 125 watt heat lamps thermostatically regulated. The lamps were placed no closer than 14 inches from the bottom of the incubator. Newspapers were used as bedding. Coarsely shredded newspaper on top of the bedding was effective in keeping the young pups clean.

The pups were fed a bitch milk replacement, Esbilac¹. An electric blender was used to suspend the milk solids in hot tap water. The reconstituted milk, prepared daily, was placed in baby bottles and sterilized in an autoclave for 20 minutes at 15 pounds pressure. Each pup had its own bottle (Fig. 53) and care was taken to prevent exposing the pups to D. canis from the handler's clothing or skin. Hands were carefully washed with soap and water before and after handling each pup.

Tissues were macerated, centrifuged, and the sediment examined by the KOH method as outlined on pages 32 and 33. Histologic preparations and methods are outlined on page 33. The technique for skin scraping, as outlined in detail by Benbrook and Sloss (1961, pages 152-161), was used for finding

¹The Borden Company, 350 Madison Avenue, New York 17, N.Y.

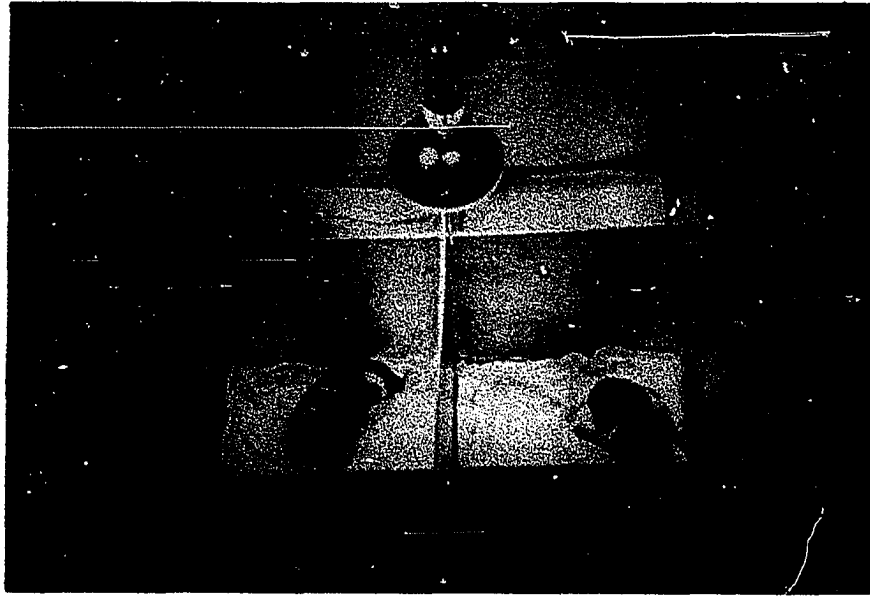


Fig. 52. Incubator with four individual compartments



Fig. 53. Feeding pup with baby bottle

living specimens of D. canis. A drop of mineral oil was placed on a clean scalpel blade. A fold of suspected skin lesion was supported with one hand and scraped with the oiled scalpel blade. The scalpel blade edge was held perpendicular to the skin to facilitate scraping and prevent cutting. Scraping was continued until blood began to flow. The skin scrapings on the oiled scalpel were transferred to a drop of mineral oil on a clean glass slide. The scrapings were covered with a coverglass and systematically examined with a microscope at 100 magnification. Failure to find D. canis by the skin scraping technique did not necessarily mean the dog was free of mites.

Skin scrapings were used for infecting pups in the dermal transmission investigations. Saline, 0.85% sodium chloride, was used instead of mineral oil for collecting the mites from skin lesions. The scrapings containing D. canis were placed in a small dish of saline and later transferred by pipette directly to the skin of the pup.

Dogs were destroyed by intravenous injections of magnesium sulfate (35 g $MgSO_4$ in 100 ml water).

INVESTIGATIONS

Dermal Transmission

Several authors (Wernicke and Stolte, 1923; Nørr, 1934; Rubin, 1957; and Koutz, 1957) have reported no transmission of D. canis by placing the mites on the skins of dogs. These negative reports may have been the result of using pups that were too old, or methods for detecting small populations of D. canis were inadequate. There is no substantiated report of transmission of D. canis to a dog more than three months old, either by placing mites directly on the skin or by living in close association with dogs having demodectic mange. Unsworth (1946) and Enigk (1949) reported successful transmission to pups less than three months old.

Unsworth (1946) reported failure to produce "clinical lesions" on 14 pups, age two to eight weeks. Mites were recovered from three pups 19, 31, and 47 days after application of material containing living D. canis at 2, 8 and 10 weeks of age.

Enigk (1949) applied lanolin, containing D. canis, to shaved areas on the backs of pups. The lanolin was covered with gauze affixed with adhesive tape. Mites were recovered from the shaved areas 12, 26, and 36 days after application to three pups aged 7, 10, and 6 weeks, respectively. Two small skin lesions were visible six months after the mites were applied to an eight-week old pup.

In order to attempt dermal transmission of D. canis to pups, mites were applied directly to the foreheads of young pups (Litters I, II, and III) or they were reared by dams having demodectic mange (Litters III, IV, and V).

Litter I

A pregnant Beagle was negative for D. canis when repeatedly examined by skin scrapings. Six pups were taken from her by cesarean section. One pup, 39D, was delivered dead and no mites were found in its skin samples when examined by the KOH method. The remaining five pups were reared in an incubator. When 24 hours old, two pups (41D and 42D) received skin scrapings, containing D. canis, on the forehead. The skin scrapings, in saline, were applied by a pipette. At 48 hours, mites in skin scrapings were again applied to the forehead of 41D and were covered with a plastic bandage (Curad¹). The 48-hour old pup, 42D, was exposed to a dog (19D) with demodectic mange. The face and forehead of the pup was rubbed and held against the diseased skin for five minutes. One pup, 43D, nursed the dam three times during the first day, then remained in isolation. Two pups, 44D and 45D, were kept in isolation from birth.

D. canis were found easily by scraping the forehead skin

¹The Kendall Company, Bauer and Black Division, Chicago, Illinois.

of 41D and 42D nine weeks after application of the mites. Unfortunately, when three months old, 41D and 42D were killed by another dog. Definite skin lesions were not visible at necropsy. However, the mite populations had apparently established and mites were found in skin samples from the eyelid, forehead, cheek, shoulder, and hip. No mites were found in skin samples (1 to 1.5 square cm in area) taken by biopsy from foreheads of 43D, 44D, and 45D at nine weeks. At three months, no mites were found by skin scraping the heads of 43D, 44D, and 45D and they were considered negative for D. canis.

Litter II

Four pups (65D, 66D, 67D, and 68D) were taken from their dam within 24 hours of their natural birth. They were reared in an incubator. Skin scrapings, containing live D. canis, were placed on the gauze of a plastic bandage (Band-aid¹). The gauze was moistened with tap water and placed between the eyes of the pups, 67D and 68D, before the pups were one day old.

A mite was found, using the KOH method, in the skin sample taken at 25 days from 67D. Eight weeks after application of the mites, one female and one deutolarva of D. canis were recovered from the forehead of 68D.

No mites were found by the KOH method in skin samples

¹Johnson and Johnson, New Brunswick, New Jersey.

from the two pups (65D and 66D) reared in an incubator without a dermal application of mites. Skin samples from the eyelid, forehead, shoulder, and hip were taken at necropsy from 66D at 25 days of age and from 65D at eight weeks. Two pups, 69D and 72D also from Litter II, were reared by the dam and were negative for mites. Skin samples from the eyelid, forehead, shoulder, and hip were taken at necropsy of 69D at 25 days of age. A skin sample, approximately 1 square cm, was taken by biopsy from 72D at ten weeks of age.

Litter III

Eight pups were delivered naturally by 13D (sire, 7D). No later than three hours after natural birth, four pups (46D, 47D, 48D, and 49D) were isolated from the dam and reared in an incubator. Mites were applied to the foreheads of 46D and 47D about five hours after birth. The mites were placed between the eyes and secured by a moist plastic bandage for two days. Two other pups, 48D and 49D, were placed directly in the incubator.

Skin samples were taken from 46D at three weeks of age. Only one mite was recovered from the forehead by the KOH method. At eight weeks mites were found in skin scrapings from the forehead of 47D. Skin samples taken by biopsy from the foreheads of 48D and 49D were negative at eight weeks. However, mites were found in skin samples from the flank of 49D at nine weeks. Mites were also present in skin samples

from the neck of 48D at 10 weeks. No mites were found in the eyelids or foreheads of 48D and 49D. Of the four pups that remained with the dam, one was destroyed at three weeks and the others were examined at eight weeks. All four pups had mites in the skin of the forehead.

The data from this litter are not readily understood. The results were interpreted as follows: (1) transmission was successful to the foreheads of 46D and 47D; (2) the few mites found on the flank of 49D and the neck of 48D were the result of the brief contact with the dam; and (3) the dam transmitted mites to the four pups reared with her.

Litter IV

Three pups were born naturally to a dam that had a mild demodectic mange, Fig. 54. The dam and sire (13D and 7D) were the same as for Litter III. The pups (21D, 22D, and 23D) were isolated from the dam about six hours after birth. Pup 21D died at age 2.5 days. Mites were found in histologic preparations of skin from the forehead, principally between the eyes (Fig. 55). Mites were found in skin scrapings from the foreheads of 22D and 23D at three months. Although neither 22D nor 23D ever had any visible lesions, mites were found in skin samples from the head at eight months.

Litter V

A litter of five pups was delivered naturally by 12D (sire unknown). Three of the pups (15D, 16D, and 17D) were

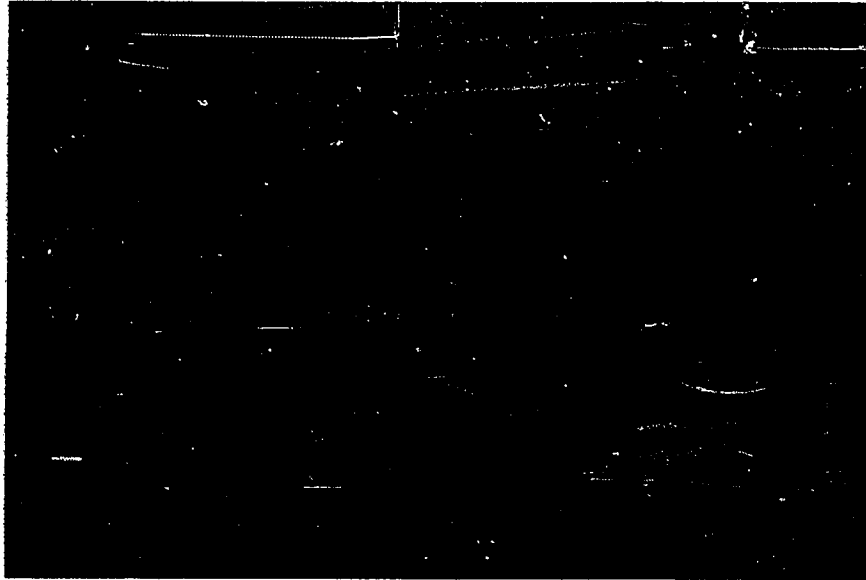


Fig. 54. Dam 13D with a mild demodectic mange two months before delivery of Litter IV



Fig. 55. D. canis (arrow) in hair follicle of 2.5 days old pup, 21D. Hematoxylin and eosin. X 200

isolated from the dam approximately 76 hours after birth and reared in an incubator. The other two, 18D and 19D, remained with the dam. D. canis was found in skin scrapings from each of the pups after two months. Fig. 56 illustrates the early lesions on forehead of 15D at two months; at four months there were no noticeable lesions. Only 19D developed a chronic demodectic mange, lasting at least 16 months (Figs. 57, 58, 59, and 60).

Litter VI

Seven pups were taken by cesarean section from 12D (sire, 7D). The dam had no visible lesions at whelping. Chronic demodectic mange of 16 months duration had apparently ended about two months before whelping.

The pups were isolated from the dam after delivery and reared in incubators. Four of the pups died at 0.5, 1, 1, and 9 days. No mites were found in skin samples from the head and neck of these four pups. Skin samples were taken by biopsy from the foreheads of the remaining three pups at 3.5 months of age. No mites were recovered by the KOH method.

One pup, aged 3.5 months, was placed in a pen with two dogs (22D and 23D) having low populations of D. canis but no visible lesions. At eight months of age this pup was destroyed. No mites were found in skin samples from the eyelid, nose, cheek, forehead, foreleg, flank, or hindleg.



Fig. 56. Lesions on forehead of 15D at two months of age.
Litter V

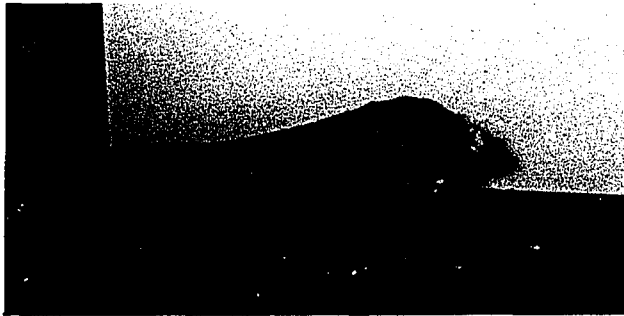


Fig. 57. Lesions on head of 19D at 14 weeks of age.
Litter V



Fig. 58. Lesions on head of 19D at 18 weeks of age.
Litter V



Fig. 59. Lesions on forehead of 19D at 8 months of age. Litter V



Fig. 60. 19D with demodectic mange at one year of age. Litter V

Prenatal Transmission

Although there are speculations in the literature (Enigk, 1949; Kirk, 1950; and Rubin, 1957) as to the possibility of infection of fetuses by D. canis, no documented experiment proving prenatal transmission has been published to my knowledge.

Observations concerning prenatal transmission were made on two litters.

Litter VII

A terrier of mixed ancestry, with a moderate squamous demodectic mange (Figs. 61 and 62), delivered seven pups. Four pups were taken immediately at birth and their placental membranes removed by hand. Great care was exercised not to contaminate the pups with D. canis from the dam. Each of the four pups was placed in a separate compartment of an incubator for hand rearing. The other three pups remained with their mother.

The four hand-reared pups were destroyed; skin samples from the forehead, shoulder, flank, and hip were carefully examined by the KOH method. All four pups (61D, 62D, 63D, and 64D) reared in the incubator, without contact with the dam after birth, were free of D. canis when examined at 24, 57, and 85 days of age. The three pups (58D, 59D, and 60D) that remained with their dam were positive for D. canis.

Two pups, 61D and 58D, were destroyed at age 24 days.



Fig. 61. Dam 56D with moderate squamous demodectic mange five days after delivering Litter VII



Fig. 62. Dam 56D with demodectic mange ten weeks after delivery of Litter VII

Eight adults, 10 ova, and 9 larvae were recovered from the forehead and shoulder skin samples of 58D. No mites were found in the mandibular lymph nodes. No mites were found in skin samples from 61D. Two other pups (62D and 63D), reared in incubator, were destroyed at 57 days of age. No mites were found by the KOH method in their skin samples. Pup 64D was also negative for D. canis when destroyed at 85 days of age.

The two pups (59D and 60D) that had remained with their dam were showing clinical symptoms of demodectic mange (Fig. 63) at 57 days of age. Three weeks later these two pups were almost hairless, emaciated, edematous, and muscularly weak (Fig. 64). In addition to a very high population of Demodex, the pups were parasitized by hookworms (Ancylostoma caninum) and ascarids (Toxocara canis). At 85 days of age, 60D died and 59D was destroyed. Mites were found (by the KOH method) in a mandibular, prescapular, superficial inguinal, and popliteal lymph node from each pup. More than a hundred specimens, including all stages of D. canis (few ova), were recovered from all four lymph nodes of 59D.

Litter VIII

A Boston terrier (54D), as shown in Fig. 65, was almost hairless at whelping. Mites were observed in a prescapular lymph node of this dam 64 days before whelping. Three pups were removed by hand from the placental membranes at birth.

No mites were recovered by the KOH method from the entire

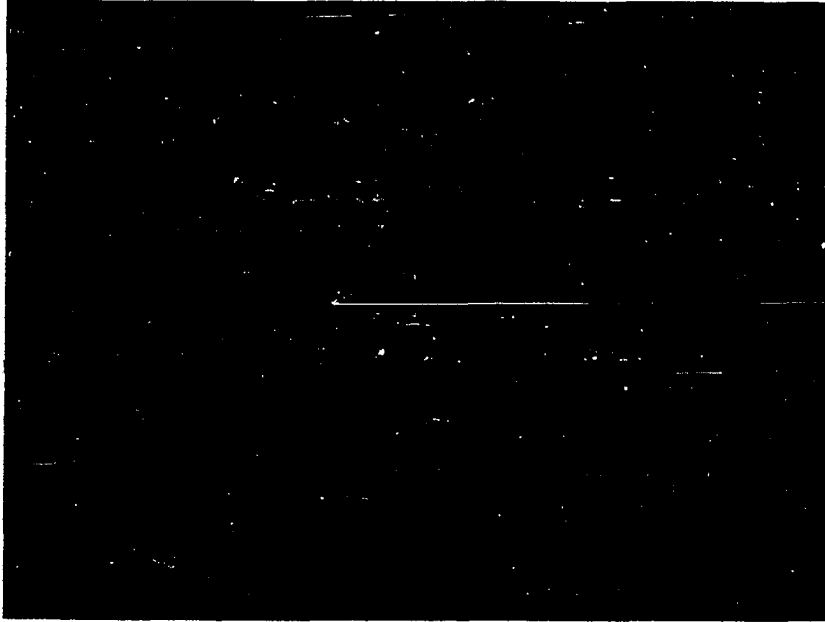


Fig. 63. Pups (59D and 60D), aged nine weeks, reared with dam 56D (Figs. 61 and 62) showing clinical symptoms of demodectic mange. Litter VII

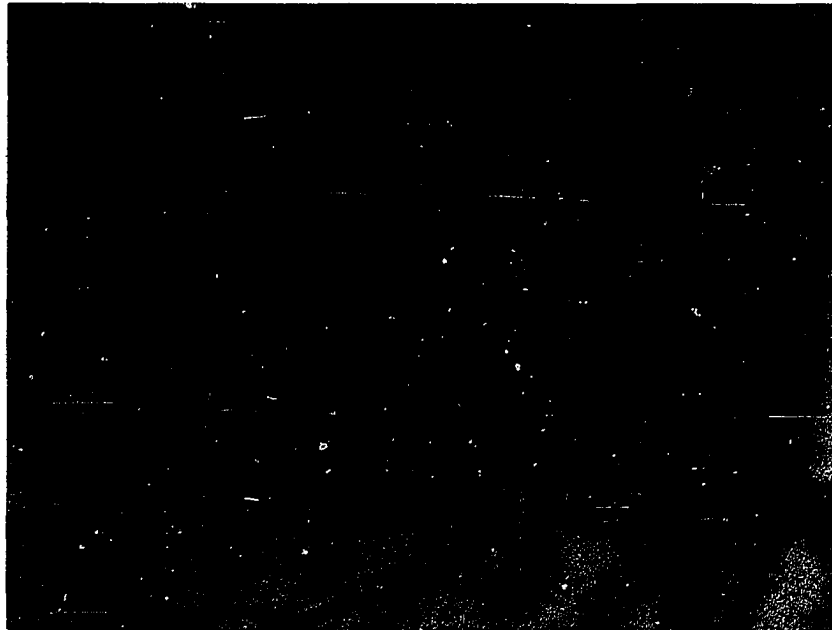


Fig. 64. Pup 59D, 85 days old. Litter VII



Fig. 65. Dam 54D, with extensive squamous demodectic mange, two days before delivery of Litter VIII

cadavers of the two pups that were delivered dead. The remaining pup, 75D, was reared in an incubator and was destroyed at three weeks of age. No mites were found in the skin or mandibular lymph nodes of 75D.

The absence of D. canis in this litter was significant. It demonstrated that a bitch with extremely advanced skin lesions, with secondary bacterial infection, and with mites in the lymphatic system could produce pups which were free of D. canis at birth. It was unfortunate that only one pup survived and a control pup could not be reared by the dam. However, in view of the ease in which mites were induced to establish populations on young pups of Litters I, II, and III, I have no doubt that any pups reared by such a bitch would have D. canis in their skin.

Peroral Transmission

Cánepa and da Graña (1945) reported peroral transmission of mites to eight pups, 1 to 3.5 months of age. Rubin (1957) stated: "I have been able to cause demodicosis by feeding and by intraperitoneal injection of infected material." Despite publication of these apparent successes, the per os route of transmission has failed to gain support from other researchers. Unsworth (1946) and Enigk (1949), in independent studies, presented evidence indicating that puppies could not be infected with D. canis by this method.

Unsworth attempted transmission to three puppies (age not

given) by feeding them each 1 square inch of skin from an active demodectic mange lesion. Another pup was fed a pre-scapular lymph node (the corresponding lymph node from the opposite side of the body contained 614 mites). No mites were found in the four pups, which were destroyed after one month. Skin samples and mandibular, prescapular, mesenteric, portal, and iliac lymph nodes were examined.

Enigk (1949) concluded that peroral transmission did not occur in 12 dogs ranging in age from 6 days to 18 months. Each dog was fed material containing living mites. The peroral inoculations varied from six feedings, of at least 100 mites each, during a four day period to 14 inoculations in 47 days. Examination techniques varied and were performed from five days to ten months after the last inoculation. Some of the dogs were examined only by skin scraping. Others were examined more thoroughly at necropsy; the skin was detached from the cadaver, moistened, rolled, and allowed to decay in a closed container for five days. The hair was then pulled from the dermis; skin scrapings were made from the epidermis and examined. Internal organs were macerated and the sediment examined for mites. No mites were found in samples of liver, spleen, lung, kidney, intestine and the following lymph nodes: bronchial, portal, mesenteric, mandibular, medial retropharyngeal, prescapular, axillary, iliac, lumbar, superficial inguinal, and popliteal.

I attempted peroral inoculation of D. canis to three pups

of Litter IX.

Litter IX

Two days after birth, five pups were removed from their dam and reared in individual compartments of an incubator. Skin scrapings in saline were given per os with a pipette to three of the pups, aged three weeks. The saline contained numerous D. canis in all stages of development. Peroral inoculation was repeated two days later.

Subsequent skin scrapings and biopsy of prescapular lymph nodes from the inoculated animals revealed no mites. The lymph nodes were macerated in 8% potassium hydroxide and the residue examined. After an observation period of six months in which no evidence of D. canis could be found, the dogs were considered to be free from the mite.

SUMMARY

The criterion used for positive transmission of D. canis to pups was the presence of living mites in the skin rather than visible skin lesions. Transmission was accomplished by direct application of skin scrapings containing D. canis to the forehead skin of six pups, 5 to 24 hours old, from three litters. After exposure to the mites, these pups were isolated and reared in incubators. Seven other pups from the same three litters were also reared by hand, not exposed to D. canis, and were free of mites at examination.

A total of eight pups from three litters were reared by dams with demodectic mange; all were positive for D. canis. Five of these pups did not have visible skin lesions and three developed demodectic mange.

Prenatal transmission of D. canis did not occur in two litters delivered from dams with demodectic mange. Four pups that were removed from the fetal membranes and reared in isolation had no mites in the skin at necropsy. These four pups were destroyed at 24, 57, 57, and 85 days. Three littermates were reared by the dam. One pup reared by the dam had mites in the skin when destroyed at 24 days of age; the other two pups showed skin lesions at 57 days. The second dam was almost hairless at whelping of three pups. Two pups were born dead. These pups were removed from the fetal membranes and the entire bodies were macerated. No mites were found upon

examination of the residue. The third pup was removed from fetal membranes and reared in isolation. This pup was destroyed at 21 days, but no mites were found upon examination of the skin and mandibular lymph nodes.

Peroral transmission was not successful when mites were fed to three pups at three weeks of age.

It was concluded that populations of D. canis could establish in the skin of young pups either after contact with diseased skin of the dams or after artificial exposure to skin scrapings containing D. canis. Transmission of the mites to pups per os or in utero was not successful.

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ACKNOWLEDGMENTS

This dissertation is dedicated to my wife, Lorraine Gustafson French, who spent many hours feeding and attending pups that were hand-reared in our home.

I appreciate the years of patient guidance by Dr. Earle S. Raun, professor in charge of study and research. This work was carried out under Project 1309, supported in part by research grant E-3412(R1) from the Allergy and Infectious Diseases Division, U.S. Public Health Service and a research grant from Dow Chemical Company, Midland, Michigan.

I am grateful to Dr. D.L. Baker, professor of veterinary medicine and surgery, for support and guidance of the transmission study; Dr. William S. Monlux, former professor of veterinary pathology, for his critique of the histopathologic observations reported herein; Dr. E.A. Benbrook, professor of veterinary pathology, for his review and suggestions concerning this manuscript; and Dr. Ellis A. Hicks, professor of zoology, for his interest and critique of the morphology study.

I gratefully acknowledge the diligent work and acute observations of Mr. Reynaldo A. Garcia, who performed far beyond the role of laboratory assistant. I am indebted to Drs. Paul A. Dahm and T.A. Brindley, professors of entomology, for making available equipment under their jurisdiction.

APPENDIX

Mounting Media

Hoyer's modification of Berlese's solution

The formula and mixing procedure were adapted from Strandtmann and Wharton (1958).

Ingredients

| | |
|--|-------|
| Water, distilled..... | 50 ml |
| Gum arabic..... | 30 g |
| Chloral hydrate ($\text{CCl}_3 \cdot \text{CH}(\text{OH})_2$)..... | 200 g |
| Glycerin ($\text{C}_3\text{H}_5(\text{OH})_3$)..... | 20 g |

Mixing procedure Add gum arabic to distilled water and heat gently in water bath. Nearly all the gum arabic should be dissolved before the chloral hydrate and glycerin are added. The resulting solution was filtered by Schleicher and Schuell analytical filter paper, shark-skin.

Polyvinyl alcoholLacto-phenol

| | |
|--|------|
| Phenol crystals ($\text{C}_6\text{H}_5 \cdot \text{OH}$)..... | 22 g |
| Lactic acid, 85% ($\text{CH}_3 \cdot \text{CH}(\text{OH}) \cdot \text{COOH}$)..... | 22 g |

PVA

| | |
|--|-------|
| Polyvinyl alcohol, 98% hydrolyzed ¹ | 20 g |
| Water, distilled, cold..... | 80 ml |

¹Obtained from Matheson Coleman and Bell Division, The Matheson Company, Inc., East Rutherford, New Jersey.

Add the polyvinyl alcohol to the cold distilled water and stir. Heat the resulting solution in a water bath and stir continuously.

Mixing procedure Add the entire lacto-phenol solution to 56 g of the PVA solution and stir.

Stains

Stain 1. Mayer's hematoxylin and eosin

This procedure was adapted from Armed Forces Institute of Pathology (1960, page 27).

Solutions

Mayer's hematoxylin

| | |
|---|-----------|
| Hematoxylin (C.I. No. 1246)..... | 1.0 g |
| Distilled water..... | 1000.0 ml |
| Sodium iodate (NaIO_3)..... | 0.2 g |
| Aluminum ammonium sulphate ($\text{AlNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$)..... | 50.0 g |
| Citric acid ($\text{H}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$)..... | 1.0 g |
| Chloral hydrate ($\text{CCl}_3 \cdot \text{CH}(\text{OH})_2$) | 50.0 g |

Stock 1% alcoholic eosin

| | |
|-------------------------------|---------|
| Eosin Y (C.I. No. 45380)..... | 1.0 g |
| Distilled water..... | 25.0 ml |
| dissolve, then add: | |
| Ethyl alcohol, absolute..... | 75.0 ml |

Working eosin solution

| | |
|-------------------------------|---------|
| Stock 1% alcoholic eosin..... | 1 part |
| Ethyl alcohol, 80%..... | 3 parts |

Staining procedure The slides were stained, using an Autotechnicon, by the following schedule:

| | |
|--|-------|
| 1. Xylene..... | 5 min |
| 2. Ethyl alcohol, absolute..... | 3 min |
| 3. Ethyl alcohol, 95%..... | 2 min |
| 4. Ethyl alcohol, 70%..... | 2 min |
| 5. Tap water..... | 2 min |
| 6. Mayer's hematoxylin..... | 2 min |
| 7. Tap water..... | 2 min |
| 8. Working eosin solution..... | 1 min |
| 9. Ethyl alcohol, 70%..... | 1 min |
| 10. Ethyl alcohol, 95%..... | 1 min |
| 11. Ethyl alcohol, absolute..... | 1 min |
| 12. Xylene..... | 2 min |
| 13. Mount in Harleco synthetic resin (HSR) ¹ , xylene solvent | |

Stain 2. Mallory's phosphotungstic acid hematoxylin for fibrin (PTAH)

This procedure was modified from Pearse (1960, page 818) and Armed Forces Institute of Pathology (1960, pages 55-56).

Solutions

Alcoholic iodine

| | |
|-------------------------|----------|
| Iodine (I)..... | 1.0 g |
| Ethyl alcohol, 95%..... | 100.0 ml |

¹Arthur H. Thomas Company, Philadelphia, Pa.

Eosochrome

| | |
|---|---------|
| Potassium chromate ($K_2Cr_2O_7$), 3%, aqueous..... | 3 parts |
| Hydrochloric acid (HCl), 10%..... | 1 part |

Acid permanganate

| | |
|---|---------|
| Potassium permanganate ($KMnO_4$), 0.5%, aqueous..... | 47.5 ml |
| Sulfuric acid (H_2SO_4), 3%..... | 2.5 ml |

Phosphotungstic acid hematoxylin

| | |
|---|----------|
| Hematoxylin (C.I. No. 1245)..... | 1.0 g |
| Phosphotungstic acid ($H_2P_2O_7$)..... | 20.0 g |
| Distilled water..... | 100.0 ml |

Dissolve the solid ingredients in separate portions of water, the hematoxylin with gentle heating. When cool, combine.

Then add:

| | |
|--|--------|
| Potassium permanganate ($KMnO_4$)..... | 0.18 g |
|--|--------|

Staining procedure

Fixation by 4% formaldehyde,

embedded in parafin, and sectioned at 10 μ .

| | |
|--|-------------|
| 1. Xylene..... | 2 min |
| 2. Ethyl alcohol, absolute..... | 2 min |
| 3. Ethyl alcohol, 95%..... | 2 min |
| 4. Ethyl alcohol, 70%..... | 2 min |
| 5. Tap water..... | 2 min |
| 6. Mercuric chloride ($HgCl_2$), saturated aqueous solution..... | 6 to 24 hrs |
| 7. Tap water, running..... | 3 min |
| 8. Alcoholic iodine..... | 10 min |
| 9. Tap water, running..... | 2 min |

| | |
|--|------------------|
| 10. Postcrome..... | 30 min |
| 11. Tap water, running..... | 3 min |
| 12. Acid permanganate..... | 2 min |
| 13. Tap water, running..... | 3 min |
| 14. Oxalic acid, 1%, aqueous..... | 4 min |
| 15. Tap water, running..... | 2 min |
| 16. Phosphotungstic acid hematoxylin..... | 12 to 24 hrs. |
| 17. Ethyl alcohol, 99%..... | 2 min |
| 18. Ethyl alcohol, absolute..... | 2 min |
| 19. Xylene..... | 2 min |
| 20. Mount in Harleco synthetic resin, xylene solvent. | |

Stain 3. Weigert's stain for fibrin

This technique was modified from Armed Forces Institute of Pathology (1960, pages 118-119).

Solutions

Lithium carmine solution

Carmine (C.I. No. 75470)..... 4.0 g
 Lithium carbonate (Li_2CO_3), saturated aqueous.....100.0 ml
 Dissolve carmine in lithium carbonate solution and boil 10 to 15 minutes. When cool add:
 Thymol ($\text{C}_{10}\text{H}_{14}\text{O}$)..... 1.0 g
 Filter before use.

Crystal violet solution A

Ethyl alcohol, absolute..... 33.0 ml

Aniline ($C_6H_5NH_2$)..... 9.0 ml
 Crystal violet (C.I. No. 42555)..... 4.5 g

Crystal violet solution B

Crystal violet (C.I. No. 42555)..... 2.0 g
 Distilled water..... 1000.0 ml

Crystal violet working solution

Crystal violet solution A..... 1 part
 Crystal violet solution B..... 9 parts
 Working solution will keep about one week

Gram's iodine solution

Iodine (I)..... 1.0 g
 Potassium iodide (KI)..... 2.0 g
 Distilled water..... 300.0 ml

Staining procedure

1. Xylene..... 3 min
2. Ethyl alcohol, absolute..... 2 min
3. Ethyl alcohol, 95%..... 2 min
4. Distilled water..... 2 min
5. Lithium carmine solution..... 2-5 min
6. 1% HCl in 70% ethyl alcohol..... 30 sec
7. Tap water..... 4 min
8. Crystal violet working solution..... 8 min
9. Drain and blot slides with filter paper
10. Gram's iodine solution..... 5-10 min
11. Drain and blot with filter paper

12. Differentiate in mixture of equal parts of aniline oil and xylene, until no more purple washes out
13. Blot with filter paper
14. Xylene, 3 changes..... 3 min each
15. Mount in HSR

Stain 4. Silver impregnation for reticulin

This technique was modified from the method outlined by Pearse (1960, pages 817-818).

Solutions

Wilder's silver bath To 5 ml of 10% silver nitrate add one drop of 28% ammonia; a brown precipitate will form. Continue adding 28% ammonia, drop by drop, until nearly all of the precipitate is dissolved. Add 5 ml of 3% sodium hydroxide. Add 28% ammonia, drop by drop, until the solution is nearly clear. Add glass-distilled water to make 50 ml of silver bath solution. All silver solutions must be made in chemically clean glassware.

Staining procedure

1. Xylene..... 2 min
2. Ethyl alcohol, absolute..... 2 min
3. Ethyl alcohol, 95%..... 2 min
4. Ethyl alcohol, 70%..... 2 min
5. Alcoholic iodine, (see Stain 2)..... 10 min
6. Tap water..... 2 min
7. Acid permanganate (see Stain 2)..... 1 min
8. Tap water..... 2 min

| | |
|---|---------|
| 9. Oxalic acid, 1%, aqueous..... | 1 min |
| 10. Water, glass-distilled..... | 1 min |
| 11. Water, glass-distilled..... | 1 min |
| 12. Ferric ammonium sulphate ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$), 2%, aqueous..... | 7 min |
| 13. Water, glass-distilled..... | 1 min |
| 14. Water, glass-distilled..... | 1 min |
| 15. Wilder's silver bath..... | 5-7 sec |
| 16. Formaldehyde (HCHO), 4%, aqueous, neutralized with CaCO_3 , crystals..... | 30 sec |
| 17. Tap water..... | 2 min |
| 18. Gold chloride (AuCl_3), 0.2%, aqueous..... | 1-3 min |
| 19. Tap water..... | 2 min |
| 20. Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$), 5%, aqueous | 5 min |
| 21. Tap water..... | 1 min |
| 22. Ethyl alcohol, 70%..... | 1 min |
| 23. Ethyl alcohol, 95%..... | 1 min |
| 24. Ethyl alcohol, absolute..... | 1 min |
| 25. Xylene..... | 2 min |
| 26. Mount in H.S.R. | |

Stain 5. Gomori's one step trichrome

The procedure was adapted from Armed Forces Institute of Pathology (1960, page 66).

Solutions

Bouin's fluid

Picric acid ($\text{C}_6\text{H}_2\text{O}(\text{HO}_2)_3$) saturated aqueous solution 75.0 ml

| | |
|---|---------|
| Formaldehyde (HCHO), 37-40% | 25.0 ml |
| Glacial acetic acid (CH ₃ COOH)..... | 5.0 ml |

Trichrome stain

| | |
|---|----------|
| Chromotrope 2R (C.I. No. 29) | 0.6 g |
| Light green SF (C.I. No. 42095)..... | 0.3 g |
| Glacial acetic acid (CH ₃ COOH) | 1.0 ml |
| Phosphotungstic acid (P ₂ O ₅ ·24WO ₃ ·XH ₂ O)..... | 0.8 g |
| Distilled water..... | 100.0 ml |

Weigert's iron hematoxylin, stock A

| | |
|----------------------------------|----------|
| Hematoxylin (C.I. No. 4246)..... | 1.0 g |
| Ethyl alcohol, absolute..... | 100.0 ml |

Weigert's iron hematoxylin, stock B

| | |
|--|---------|
| Ferric chloride (FeCl ₃ ·6H ₂ O) 29%, aqueous..... | 4.0 ml |
| Distilled water..... | 95.0 ml |
| Hydrochloric acid (HCl) 37-38%, concentrated..... | 1.0 ml |

Weigert's iron hematoxylin, working solution

Equal parts of stock A and stock B

Staining procedure

| | |
|----------------------------------|-------|
| 1. Xylene..... | 2 min |
| 2. Ethyl alcohol, absolute..... | 2 min |
| 3. Ethyl alcohol, 95%..... | 2 min |
| 4. Tap water..... | 2 min |
| 5. In Bouin's fluid at 56°C..... | 1 hr |
| 6. Tap water, running..... | 3 min |

| | |
|--|--------|
| 7. Weigert's iron hematoxylin, working solution... | 10 min |
| 8. Tap water..... | 2 min |
| 9. Trichrome stain..... | 15 min |
| 10. Acetic acid, 0.5%..... | 2 min |
| 11. Tap water..... | 1 min |
| 12. Ethyl alcohol, 95%..... | 1 min |
| 13. Ethyl alcohol, absolute..... | 1 min |
| 14. Ethyl alcohol, absolute..... | 1 min |
| 15. Xylene..... | 2 min |
| 16. Mount with H.S.R. | |